



Lasso peptides realm: Insights and applications

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ABSTRACT

Lasso peptides exhibit a range of bioactivities, including antiviral effects, inhibition of the glucagon receptor, blockade of the endothelin type B receptor, inhibition of myosin light chain kinase, and modulation of the atrial natriuretic factor, as well as notable antimicrobial properties. Intriguingly, lasso peptides exhibit remarkable proteolytic and thermal stability, addressing one of the key challenges that traditional peptides often face. The challenge in producing those valuable peptides remains the main hurdle in the way of producing larger quantities or even modifying them with more potent analogues. Genome mining and heterologous expression approaches have greatly facilitated the production of lasso peptides, moving beyond mere isolation techniques. This advancement not only allows for larger quantities but also enables the creation of additional analogues with improved stability and potency. This review aims to explore the unique bioactivities and stability of lasso peptides, along with recent advancements in genome mining and heterologous expression that address production challenges and open pathways for engineering potent analogues.

1. Introduction

Peptides are the final products of the central dogma of molecular genetics, which involves DNA transcription and mRNA translation to produce proteins [1]. These proteins can be modified during or after the translation process, known as co-translational or post-translational modifications (PTMs), respectively [2]. While the genetic code translation produces ribosome-dependent peptides, PTMs can occur afterward to further modify them, as seen in lasso peptides. Lasso peptides belong to the class of ribosomally assembled and post-translationally modified peptides (RiPPs), distinguished by their characteristic "lasso" structure. This structure resembles a loop or lariat, where the peptide forms a ring with its tail threaded through, creating a knotted or noose-like appearance. Lasso peptides most closely resemble molecules like cyclotides [3], knottins [4], and defensins [5], in which disulfide bonds, along with head-to-tail cyclization in cyclotides, restrict the polypeptide chain into a unique and stable topology. Lasso peptides exhibit a wide spectrum of biological activities, including enzyme inhibition, as well as antibacterial, antiviral, and anticancer properties. With their distinctive knot-like structure, lasso peptides have emerged as highly modifiable biomolecules, lending themselves well to redesign efforts. Furthermore, they provide greater thermal and proteolytic stability, addressing one of the major inherent limitations of conventional peptides [6,7].

Lasso peptides can accommodate multiple amino acid substitutions, with variations in both the macrolactam ring and tail regions often having minimal impact on their overall biological activity. Notably, their three-dimensional structure, largely defined by the macrolactam ring, plays a crucial role in reserving their functionality, with tail modifications often well tolerated. Examples like sungsanpin [8] and chaxapeptin [9,10] demonstrate inhibitory effects against human lung cancer cells, despite variations in their tail regions. While the lasso motif contributes to peptide rigidity and stability, recent research suggests that specific residues, rather than the overall motif, are critical for their biological function [11,12]. The unique topology, along with remarkable stability and bioactivity of lasso peptides, has garnered significant interest in fields such as drug discovery and biotechnology.

Cleaving lasso peptides can create valuable catenanes—mechanically interlocked molecules (MIMs) [13]. Due to their threaded structure, lasso peptides are ideal for this purpose [13]. MccJ25 was used to produce [3]catenane and [4]catenane structures that self-assemble under moderate conditions, such as room temperature and atmospheric oxygen [13].

Advances in genome mining have unveiled new possibilities for engineering lasso peptides with enhanced pharmacological properties. For instance, studies on capistrucin revealed that many positions within the peptide structure can be substituted without disrupting its lasso formation [14], offering new avenues for derivative design. Moreover,

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natural product isolation and genome mining have uncovered lasso peptides featuring additional post-translational modifications (PTMs), such as phosphorylation [15], C-terminal methylation [16,17], glycosylation [18] and disulfide bond formation [19], expanding their functional diversity.

Recent computational advancements have further propelled lasso peptide research. Software tools like LassoHTP, developed for high-throughput prediction and design [20], and innovative algorithms for distinguishing between threaded and non-threaded precursors [21], are enabling more efficient exploration and design of lasso peptides [22]. These advancements hold promise for the development of novel lasso peptide analogues with improved stability and therapeutic potential.

Peptides have demonstrated their effectiveness in treating challenging diseases. For example, a recent peptide-based drug was approved for the treatment of Rett syndrome, marking a pioneering advance as a first-in-class therapy for this condition [23,24]. Due to their larger footprint compared to small molecules and their superior penetration capabilities—features often lacking in large biologics—interest in these valuable molecules has been growing. This increased interest is highlighted by the approval of over 100 peptide-based drugs [25–29]. Peptides possess high specificity, tolerable safety profile, ability to transduce and carry other molecules ranging from small molecules to large proteins [30]. The peptide market is experiencing significant growth, valued at \$10,000 billion in 2023 and projected to reach \$106,000 billion by 2033, with a compound annual growth rate (CAGR) of 10.8 % [31]. Glucagon like peptide-1 (GLP-1) peptides are generating the highest revenue due to their multifunctional role in treating diabetes, obesity, and their demonstrated benefits in cardiovascular diseases [32].

This review focuses on the valuable class of lasso peptides, exploring their structure, synthesis, and potential applications.

2. Lasso peptide structure

Lasso peptides are short peptides consisting of 16–21 amino acid

residues, with an isopeptide bond linking the *N*-terminus to the side β/γ -chain carboxyl group of Asp or Glu in position 8 or 9 [33]. The *C*-terminus threads through the newly formed ring (Fig. 1).

Initially, sungsanpin [8] and chaxapeptin [9], both with 15 residues, along with astexin-1, containing 23 residues [34], were considered the size extremes of lasso peptide, falling outside the typical range of 16–21 residues. However, a recent work expanded this range to 24 residues with the heterologous expression of astexin-3, [35], a similar extension with pseudomycoidin has been also achieved [18]. The longest known lasso peptides, pandonodin [36] and ubonodin [37], consist of 33 and 28 amino acids, respectively. Interestingly, the shortest known lasso peptide to date is a 13-mer called huascopeptin [38]. Therefore, the new range of lasso peptides spans 13–133 amino acids.

Lasso peptides are classified into three subclasses, class II lacking a disulfide bridge, the structure is instead stabilized by the bulky side chains of the *C*-terminal tail, whereas class I and III comprise two and one disulfide bridges for this purpose, respectively. In which, those disulfide bridge/s connect the ring with the tail region. Class IV of lasso peptides has been identified as well [36,39,40]. This class does possess a disulfide bridge; however, unlike classes I and III, this disulfide bridge does not connect the ring with the tail region but is located solely within the tail region. This arrangement results in a greater bulkiness than any side chain of proteinogenic amino acids, thereby conferring high stability to the lasso peptide knot structure (Fig. 1). In class I and III, Cys is located at position 1 of the *C*-terminal tail [41,42], and when it is replaced with Gly, then the class is either II or IV [43–47]. Interestingly, advancements in genome mining and heterologous expression have revealed various amino acids at position 1 of the *C*-terminal, such as Ser or Ala in streptomycin [48,49]; riculamin and alboverticillin [50]; caulonodin IV–VII [51]; paeninodin [52]; cattlecillin [53]; Asp in brevunin [54]; and Trp in fuscadin [55].

The role of a conserved Thr residue -at penultimate position- in the leader peptide of lasso peptide precursors has been debated, particularly regarding whether it serves solely as a recognition element for the lasso peptide maturation machinery or if it can be effectively substituted by

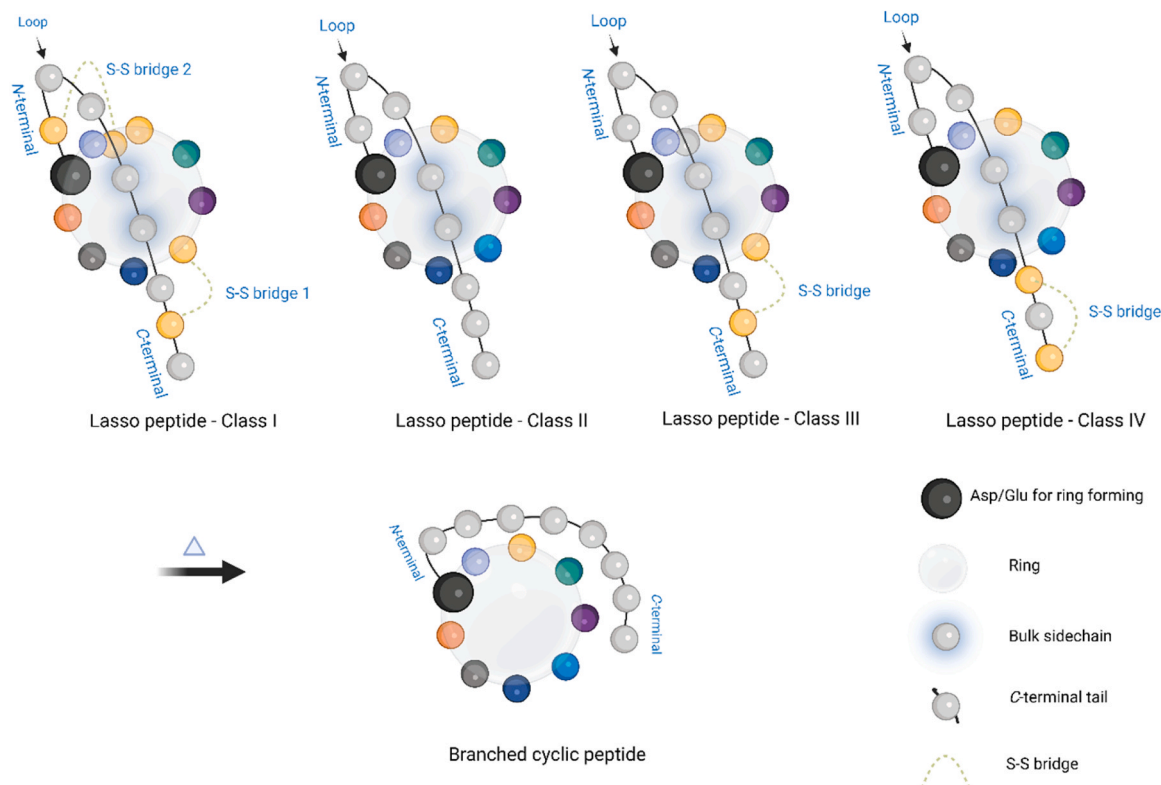


Fig. 1. Schematic representation of different classes of lasso peptides and branched cyclic peptides (unthreaded lasso peptide) topology.

other amino acids with similar size and shape. Several studies have confirmed its role as a key recognition element, aiding in the docking of the precursor within the maturation machinery [56,57]. In contrast, the xanthomonin system exhibits significantly lower tolerance to substitutions at penultimate position [58].

In paeninodin, substitution of Thr2 with another amino acid negatively impacts lasso peptide production, although structurally similar residues are better tolerated [15]. Paeninodin has been explored using *in vivo* production, *in vitro* processing, and binding studies [59]. The study confirmed that PadeB2 tolerates position 1 substitutions but is sensitive to changes at Thr2, and the YxxP motif is essential for recognition element (RRE) binding [59]. Substitutions reduce, but don't eliminate, the efficiency of lasso peptide maturation steps, showing the complex interplay between proteins and precursor peptides [59].

An insight into the complex maturation process of MccJ25, concluded that the leader sequence of the precursor McjA does not promote prefolding of the peptide [60]. The study highlights the crucial role of the maturation machinery in shaping the precursor into its lassoed form [60]. To explore RRE domains, a p-nitroanilide-based leader peptidase assay has been introduced. The analysis showed that RRE domains both render the precursor peptide to the leader peptidase and contribute to forming the S2 proteolytic pocket, which binds the penultimate Thr of lasso leader peptides [61].

3. Lasso peptides biosynthesis

The production process of lasso peptides involves the following steps: (i) a precursor peptide A, where a RiPP RRE binds to and recognizes a specific sequence in the N-terminal leader region (ii) cysteine protease B, the RRE then recruits a leader peptidase, which cleaves the precursor, releasing the C-terminal core peptide, and (iii) Adenosine triphosphate (ATP)-dependent lactam synthetase C, where a macrocyclase processes the core peptide by activating the ring-forming β - γ -carboxy side chain of either Asp or Glu, respectively, in an ATP-dependent manner. It then facilitates the nucleophilic attack of the N-terminal primary amine on the activated adenosine monophosphate (AMP) ester, leading to the formation of the macrolactam through a straightforward condensation reaction, creating a bond known as the isopeptide bond [55,59,62–65]. Then, the C-terminal tail is threaded through the formed ring and the resulted "lariat knot" structure, which is the basis of their name. It is important to note that prefolding occurs during maturation, as threading cannot take place after the ring is formed [33,66–69]. Interestingly, it was demonstrated that two enzymes, B for processing and C for cyclization, are needed to assemble the lasso peptide's knot-like structure [70]. The study showed that ATP is not necessary for the interaction between B1 and B2 proteins or for protease activity [70]. These findings differ from those in the microcin J25 system, where McjB-catalyzed cleavage requires ATP [65]. Phylogenetic analysis of B and C homologs reveals two clades: Clade I with isopeptidase-associated clusters and Clade II with "biosynthesis only" and ABC transporter clusters [71]. Further research is required to explore whether other clades employ different maturation strategies [70].

The structure is stabilized by the steric hindrance between the ring and strategical amino acid residues with bulky side chain of the C-terminal tail, known as plugs [66,72–76]. The N-terminal often comprises the following amino acids, Gly, Ala, Ser, or Cys, and the ring is usually composed of eight to nine amino acid residues (Fig. 1). Some lasso peptides, such as xanthomonins I–III [58] and huascopeptin [38], have been identified with a notably small seven-residue ring. Huascopeptin, in particular, stands out as the shortest lasso peptide discovered to date, consisting of only 13 amino acids [38]. Xanthomonins I–III demonstrated complete resistance to both proteolytic and thermal degradation [58]. No lasso peptides with fewer residues have been reported. This is because more than nine amino acids are generally considered too large to maintain the steric constraints required for the unique lasso structure, while fewer than eight residues are too small to allow proper threading.

For the small macrolactam ring in xanthomonins I–III, it appears that glycine residues are crucial for threading amino acids through the ring. The substitution of Gly10 and Gly11 with larger amino acids like Ala, Leu, or Phe, has been investigated. The findings revealed that replacing either glycine significantly hindered lasso peptide production, highlighting the critical role of glycine in maintaining the lasso structure [58]. Interestingly, Gly11 is also present in huascopeptin, further supporting the essential role of glycine in maintaining the structural integrity of lasso peptides with small macrolactam rings. Additional mutants have been created to determine if altering the size of the macrolactam ring was feasible by substituting Glu with Asp or replacing Glu7 with Ala. In addition, given the potential importance of Pro for prefolding due to its rigidity, the study examined the role of Pro3 in the maturation process [58]. The outcome indicates that the biosynthetic machinery is likely restricted to processing 23-atom macrocycles [58].

Fig. 2 illustrates the machinery responsible for the biosynthesis, maturation, and export of various lasso peptides, along with their structural genes that direct the production [65]. The precursor peptide A contains the N-terminal leader sequence. The gene products of B and C are essential for its conversion into the mature lasso peptide. A dedicated export system, encoded by gene D, provides self-immunity through active efflux of the peptide. Gene D shows structural characteristics typical of the bacterial ATP-binding cassette (ABC) transporter family and shares strong similarities with other ABC transporters [77].

Homology analysis has shown that mcjC and capC share similarities with asparagine synthetase B (AsnB) from different bacteria and contain conserved AsnB motifs involved in coordinating Mg^{2+} for ATP binding [78,79]. These enzymes are likely involved in activating the γ - β -carboxyl sidechain of the Glu or Asp residues, respectively, which are crucial for forming the isopeptide bond that closes the ring in MccJ25 and capistruin. Furthermore, mcjB and capB exhibit homology to transglutaminases and are thought to function as peptidases that cleave the leader peptide [80]. In addition, it contains a catalytic triad composed of cysteine, histidine, and aspartate, functioning as a cysteine protease. This enzyme is thought to be involved in peptide prefolding, as its catalytic activity depends on ATP consumption [76]. AlphaFold2 was considered to model the structure of the precursor McjA in association with its biosynthetic enzymes McjB and McjC. The structural and molecular aspects of MccJ25 biosynthesis have been examined, following the path of the final lasso peptide from the leader peptide through protease processing to its final maturation [81].

The lariat biosynthetic gene cluster includes a further gene, *larC*, located upstream of *larD*, which encodes a putative protease [82]. Deletion and complementation experiments have been conducted to investigate the function of *LarC*. Interestingly, *larC* is unique to the lariat gene cluster in Gram-positive bacteria, as no corresponding protein is found in the microcin J25 or capistruin biosynthetic clusters of Gram-negative bacteria. Although its precise role is not fully understood, *larC* has been demonstrated to be crucial for the production of lariat [82]. Later, it was discovered that Tyr6, Gly11, and Asn14 are crucial for lariat activity. Tyr6 and Asn14 interact closely, and altering these residues disrupts their interaction, impacting functionality. Asn14 also serves as a "plug" to stabilize the tail region in lariat A. Substituting Asp with Ala changes the peptide's conformation and diminishes its activity. These residues are key to interacting with the target in *M. smegmatis* [83].

Recent advancements in bioinformatics have greatly facilitated the discovery of numerous new ribosomal natural products, such as lasso peptides. These developments have been discussed in detail [80] and can be briefly summarized as follows:

1. Classical genome mining of RiPPs: This approach involves searching for conserved RiPP tailoring enzymes, such as Ser/Thr dehydratases, which are notably conserved within certain RiPP classes. Benchmark enzymes serve as seed sequences to identify homologs using

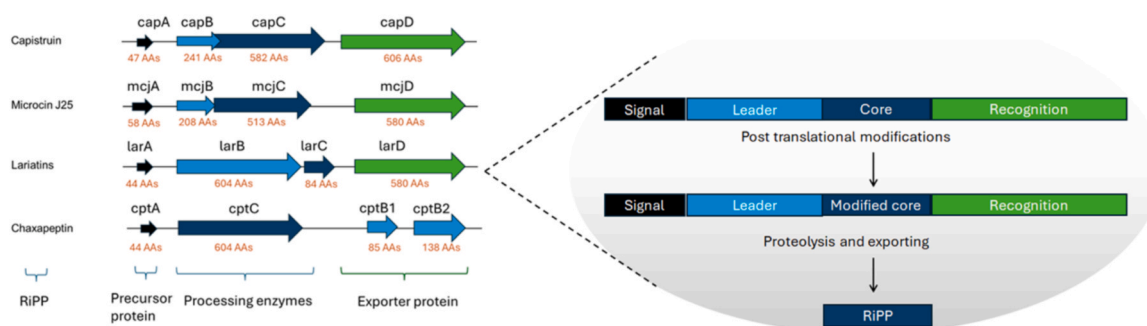


Fig. 2. Schematic representation of the biosynthesis gene clusters for selected lasso peptides, their size, functions, and the pathway of RiPP biosynthesis. The RRE binds the *N*-terminal leader sequence, recruits cysteine protease B to cleave the precursor, and ATP-dependent lactam synthetase C activates β - γ -carboxy side chain of Asp/Glu for macrocyclization.

sequence-based comparison tools like basic local alignment search tool (BLAST).

2. Mining biosynthetic gene clusters (BGCs): This method involves searching for both PTM enzymes and precursor peptides. Although precursor peptides share the same PTM enzymes, their variation can significantly expand the chemical space of RiPPs.
3. Utilizing machine learning: Tools such as RiPPMiner utilize machine learning classifiers, like support vector machines (SVM), to distinguish RiPP precursors from non-precursor peptides and predict cleavage sites. NeuRiPP is another recent AI-driven method based on deep learning, leveraging artificial neural networks for RiPP mining.
4. BGC comparison and clustering by similarity networks: Comparative BGC analysis serves as a post-processing workflow to prioritize candidates and avoid the rediscovery of known RiPPs.

These developments have spurred the rapid evolution of genome mining strategies. However, readers are encouraged to look up their paper for more comprehensive insights [84].

4. The absence of synthetic pathways

Currently, there is no synthetic method for producing lasso peptides, so their production relies on bacteria that contains the gene cluster responsible for encoding the maturation enzymes. All currently known gene clusters for lasso peptides indicate a ribosomal origin [1,26]. Additionally, mutagenesis studies of MccJ25 [85] and capistrin [14] have demonstrated the high promiscuity of the biosynthetic machinery, which enables the *in vivo* production of variants through precursor protein mutagenesis. No lasso peptide has been synthesized to date using solid-phase peptide synthesis (SPPS) due to the folding challenges involved. Standard SPPS is unlikely to produce lasso peptides solely, unless followed by *in vitro* reaction [86], or solution phase reactions [87]. The absence of synthetic routes for lasso peptides can be attributed to several factors related to their unique structural properties and synthesis challenges. These include the complex structures requiring PTMs and the conformational constraints resulting from their final structures. For instance, conventional SPPS may face challenges such as steric hindrance and solubility issues, particularly due to the inherent hydrophobicity of lasso peptides, leading to problems with aggregation and solubility.

Interestingly, A cryptand-imidazolium complex was developed as a multi-linker support for the chemical synthesis of the lasso peptide BI-32169 [88]. Both the L- and D-enantiomers of BI-32169 were synthesized with high purity of 98.7 % [88]. Furthermore, the D-enantiomer exhibited strong glucagon receptor antagonist activity and showed significantly greater stability in human serum stability assays at 95 °C compared to the L-lasso peptide [88].

In our laboratory, we have developed a new version of Merrifield SPPS, termed SPPS 2.0, which facilitates the synthesis of first-in-class

peptides [89]. We have successfully created what we refer to as cross-linked peptides, with three chains linked together on a single bead. Utilizing our technology along with appropriate linkers, we envision the potential to assemble the intriguing class of lasso peptides by cyclizing two chains and threading the third one.

5. Thermal and proteolytic stability

Due to their unique structure, lasso peptides exhibit exceptional thermal and proteolytic stability [74,76,90]. However, this property is not intrinsic for all lasso peptides and is much dependent on the peptide itself. Hence, in some lasso peptides, thermal exposure can convert the knotted structure of lasso peptide to a branched-cyclic topology (Fig. 1) [91,92]. For instance, caulosegnins I and III are an example of the first identified heat-sensitive lasso peptides [93]. The smaller the ring, the more readily a heat-stable lasso fold can be achieved. Moreover, incorporating bulkier side chains enhances the overall stability of these threaded [1]rotaxane structures. In some instances, a heat-labile lasso peptide can be transformed into a heat-stable one by substituting the native plug amino acid to a larger residue, such as Trp [51,74,76,91,92,94]. Thus, the thermal stability of caulosegnins II's is mainly attributed to the presence of Pro8 within the nine-membered ring, which helps maintaining the lasso fold [92]. In xanthomonins I–III analogues, with Ile, Leu, Thr, or Val at position 12 remained heat-stable, while those with Ala or Ser formed branched cyclic peptides. This indicates that residues bulkier than Ser maintain the lasso fold, even at high temperatures [58]. It was also proven that the highly thermolabile rubrinodin lasso peptide could be made more thermostable by incorporating bulky plug residues like Trp, which effectively stabilized the lasso structure [95]. Interestingly, the unusual residues at the *N*-terminal position 1 of the core peptide of some lasso peptides contributed to their thermostability, such as the Asp in brevunsin [54] and Ser or Ala in caulonodin IV–VII [51]. In contrast, fuscadin contains a Trp residue at its *N*-terminus; however, it has been observed to undergo unthreading in organic solvents at room temperature [55].

Benenodin-1 is able to switch between two distinct threaded conformations upon heating [96]. Two variants of benenodin-1 were designed, each with a single amino acid substitution that favors one of the conformations when heated [96]. The benenodin-1 isopeptidase can only recognize and cleave one of these conformers, offering new insights into how isopeptidases recognize their substrates [96]. The study concluded that heating causes benenodin-1 to reach an equilibrium between two unique slip knotted conformers, a novel observation for lasso peptides. The study also demonstrated that single amino acid substitutions in the plug amino acids can lead to three outcomes upon heating: irreversible unthreading, preference for the native conformer, or preference for the switched conformer [96]. Given its built-in conformational complexity and loop-cleavage capability, benenodin-1 is an excellent candidate for developing complex peptide-based

molecular machines like catenanes [96]. It was also demonstrated that benenodin-1 functions as a four-state molecular shuttle when activated by heat. The wheel motion occurs through a series of loop and tail pulling equilibria, leading to the formation of four distinct stable translational isomers [97]. Recently, benenodin-1 was modeled using a combination of QM and MM MD calculations to elucidate the effects of particular mutations on the peptide's conformational population as observed experimentally [98]. The analysis concluded that Gln15 contributes the most to the increase in 1D entropy values, revealing a significant rise in conformational flexibility in conformer 2. Additionally, Glu14 showed the highest increase in 2D mutual information values between conformer 1 and conformer 2, reflecting its enhanced correlated motions with other residues in the peptide [98]. The substitution of Glu14 to Ala favors conformer 2 from an entropic perspective, while the Gln15 to Ala mutation favors conformer 1, which aligns reasonably well with experimental observations [98].

A branched cyclic peptide, or unthreaded lasso, can be distinguished by HPLC due to the exposure of the unthreaded C-terminal tail to the solvent, which is no longer shielded by the lactam ring. Furthermore, carboxypeptidase is used for a definite differentiation between both topoisomers. Carboxypeptidases are enzymes that sequentially cleave most of C-terminal residues of a peptide until the peptide is either fully degraded or further degradation is inhibited. In the case of a lasso peptide, the isopeptide bond connecting the β - γ -carboxy side chain to the N-terminal primary amine creates a structural barrier that halts carboxypeptidase activity. This feature allows lasso peptides to be distinguished from branched-cyclic peptides. While carboxypeptidases can fully or almost completely remove the C-terminal tail of a branched-cyclic peptide, the threaded structure of a lasso peptide effectively shields its C-terminus, preventing carboxypeptidase-mediated proteolysis [10,18,36,39,52,63,91,99].

For an in-depth exploration of the factors influencing the thermal stability of lasso peptides, readers are encouraged to refer to Hegemann's recent review on this topic [74].

6. Characterization techniques

A variety of techniques can be utilized to characterize lasso peptides, including Fourier transform ion cyclotron resonance (FTICR) mass spectrometry [100], NMR spectroscopy [101], tandem mass spectrometry [102,103], atomic-resolution crystal structure [104], ion mobility–mass spectrometry [105]. In the ion mobility cell, ions drift through a buffer gas under an electric field, separating based on their collision cross section [106]. This method effectively differentiates lasso peptides from branched-cyclic topoisomers, as they adopt distinct gas-phase conformations compared to those in solution (Fig. 3) [105].

High charge states allow for distinguishing between lasso and branched-cyclic topoisomers, with lasso peptides remaining compact in the gas phase while branched-cyclic ones unfold. Three key trends set lasso peptides apart: first, they exhibit minimal change in collision cross section (CCS) as the charge state of multiply protonated molecules increases, resulting in a narrow CCS range. Second, even with

supercharging agents, highly charged protonated lasso peptides show low intensity. Third, lasso peptides display narrow ion mobility signals and small peak widths, indicating a limited number of conformations across different charge states.

Other techniques are also being developed and used including, native nanoelectrospray ionization-trapped ion mobility spectrometry–mass spectrometry (nESITIMS-MS) [107]. A study on MccJ25 confirmed that TIMS-MS and site-directed mutagenesis significantly improve our understanding of Pro residues by offering detailed insights into receptor-binding dynamics and biological activity [108]. Using high-energy collisional dissociation (HCD), the macrocycles can be opened and the newly linearized peptides fragmented, allowing for the confirmation of the complete amino acid sequences of the characterized lasso peptides [109].

7. Biological activities

Lasso peptides have diverse interesting biological activities including antimicrobial, antiviral, enzymes inhibition, and anticancer activities. Prior to 2008, all the lasso peptides were discovered by activity-driven isolation experiments [44,110]. Whereas, since 2008, genome mining approaches have been employed, showing that lasso peptides are broadly distributed across the bacterial kingdom [34,68,69,84,111] (Fig. 4). Between 1954 and 2007, there were 130 published journal articles (excluding reviews). Since 2008, and up to the time of writing this review, 148 additional articles have been found using SciFinder.

Capistrui marked the first lasso peptide to be reported by this approach [77]. The folded structure is often responsible for the biological activity of lasso peptides and therefore, it is of utmost importance to make sure that the newly isolated lasso peptides retain the correct folding [44]. The Rapid ORF description and evaluation online (RODEO) tool has been reported as a powerful genome-mining resource, used to comprehensively map lasso peptides [40]. For instance, rare lasso peptide biosynthetic gene clusters have been identified in previously unexplored phyla [40]. These studies have also identified lasso peptides with unique structural features, including one with a novel handcuff-like topology and another with a citrulline modification, which is exceptionally uncommon in bacteria [40]. This research will significantly advance our understanding of lasso peptides and provide a foundation for future genome-mining studies.

This review will examine the majority of reported biologically active lasso peptides, focusing on their structure and activities, and will include summary tables with their peptide sequences, associated indications and mode of action.

7.1. Enzyme inhibitors lasso peptides

Lasso peptides have emerged as a fascinating class of molecules with potential applications as enzyme inhibitors, regulating various physiological processes (Table 1).

Anantin acts as an antagonist to the atrial natriuretic factor (ANF) receptor from bovine adrenal cortex [43]. MS-271 inhibits myosin light

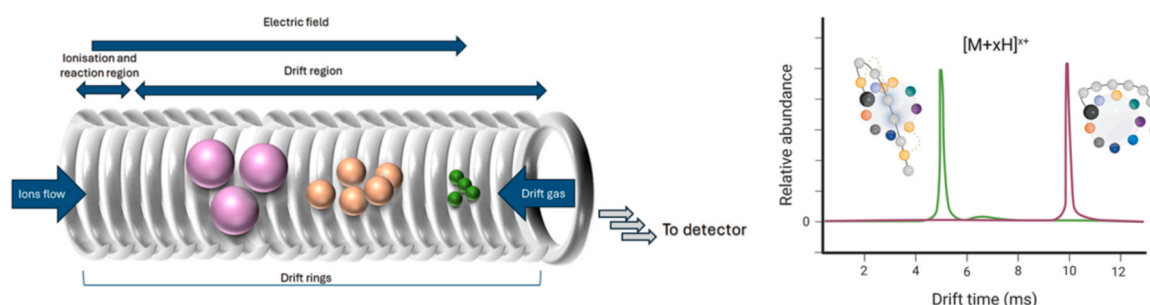


Fig. 3. Schematic representation of ion mobility spectrometer.

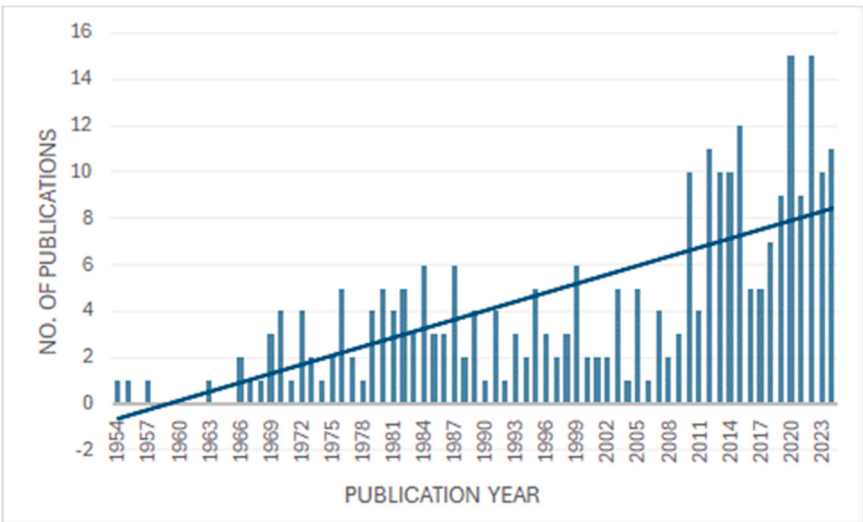


Fig. 4. Number of published articles found on SciFinder before and after the incorporation of genome mining approaches.

Table 1
Summary of the enzyme inhibitors lasso peptides.

#	Lasso peptide	S-S bridges	Sequence	Mode of action	Ref
1	Anantin	0	RFIGWGNDIFGHYSGD F	Binds to the atrial natriuretic factor (ANF)	[43]
2	MS-271	2	CLGVGSCNDFAGCGYA IVCFW	MLCK inhibitor	[110]
3	Propeptin	0	GYPWWDYADLPGGHT FISP	Inhibits prolyl endopeptidase	[47]
4	BI-32169 and its methyl ester	1	GLPWGCPSPDIPGWNT PWAC	Glucagon receptor antagonist	[16]
5	RES-701-1 RES-701-2 RES-701-3 RES-701-4	0	1: GNWHGTAPDWFFNYY W 2: GNWHGTAPDWFFNYY(7-hydroxy)W 3: GNWHGTSPDWFFNYY W 4: GNWHGTSPDWFFNYY(7-hydroxy)W	Endothelin B receptor antagonist	[46, 112]

Red, isopeptide bond between N-terminal and either β -/ γ -carboxy of Asp/Glu; green, modified C-terminal; NR, not reported; Ac, acetyl.

chain kinase (MLCK), which is responsible for smooth muscle contraction in the presence of calcium. Consequently, inhibiting MLCK is of therapeutic interest for potential use as vasodilators and bronchodilators [110]. Interestingly, MS-271 has a D-Trp within its C-terminal tail, with the rest of amino acids of L configuration [110]. A D-tryptophan modified MS-271 has been successfully engineered by identifying the gene cluster responsible for incorporating the D-amino acid [113]. The study demonstrated through heterologous expression that the epimerization of D-Trp is catalyzed by a novel epimerase encoded within the msl cluster [113]. It was also found that MslH uses acid/base chemistry for the reversible epimerization of C-terminal Trp21 in MslA [114]. This process involves two His/Asp catalytic pairs and a Ca(II) ion coordinated via water molecules [114]. RES-701 intriguing analogues function as selective antagonists of the endothelin B receptor, indicating their therapeutic potential for cardiovascular disease, renal disease, and

asthma [46,112,115,116]. Propeptin inhibits prolyl endopeptidase of the genus *Flavobacterium*, which are responsible to degrade Pro-containing peptides such as vasopressin [29], oxytocin [29], bradykinin [117], among others [47]. This inhibition helps preserve the integrity and functionality of these important peptides. BI-32169 is a 19-mer bicyclic peptide with the characteristic of lasso peptides [16]. This feature is attributed to its ring-penetrating residue pair, which is positioned four amino acids C-terminal to the Asp residue responsible for ring closure [104]. The structure of BI-32169 was confirmed through an atomic-resolution crystal structure, which supports the fundamental principles of the lasso fold and is consistent with the NMR solution structures [104]. This structure is present in both BI-32169 and its methyl ester derivative [16]. It was isolated from the culture broth of *Streptomyces* sp. (DSM 14996) and functions as a strong glucagon receptor antagonist [16]. Tandem mass spectrometry and NMR

spectroscopy revealed that BI-32169 has a specific 3D structure, classifying it as a lasso-structured peptide in the new class III of lasso peptides [102].

7.2. Antibacterial lasso peptides

A plethora of lasso peptides are identified as potent anti-bacterial agents. This review will discuss some of these intriguing classes, with the remaining examples listed in Table 2. Lasso peptides generally target essential bacterial processes, such as: (i) RNA polymerase inhibition as seen in microcin J25 (MccJ25) [45], this can halt bacterial transcription, (ii) disruption of cell membranes by interacting with bacterial membranes, leading to leakage of intracellular contents and cell death, and (iii) targeting specific bacterial receptors, where they may bind to particular transport systems or proteins on the bacterial surface [17], inhibiting vital functions.

Microcin J25 (MccJ25), one of the most well-known lasso peptides. Microcin J25 inhibits bacterial transcription by binding to and blocking the nucleotide uptake channel of bacterial RNA polymerase (RNAP) [45, 118]. Microcin J25 crosses the outer membrane by utilizing the ferri-chrome outer membrane transporter/phage receptor (FhuA) iron siderophore transporter [45,130,131]. After crossing the membrane via other proteins such as Smb, TonB, ExbD, and ExbB, it functions as an RNAP inhibitor and triggers the production of superoxide [132], with Tyr9 is considered crucial for inhibiting respiration by inducing superoxide overproduction [133]. Recent studies of MccJ25 bound to the outer membrane receptor FhuA revealed that His5 is crucial for MccJ25's binding to the FhuA pocket [45,130]. Interestingly, as the determination of lasso peptide structure could be tricky, this peptide was first identified as head to tail cyclized peptide [45,119–121]. However, subsequent studies on both the native microcin and peptides derived from acidic or enzymatic cleavage have confirmed its lasso peptide structure, including through IT-MS CID analysis and three-dimensional solution structure determination [119–122]. This type of structure, previously observed in enzyme inhibitors from *Actinobacteria*, aligns with MccJ25's ability to inhibit RNAP (Fig. 5) [45]. MccJ25 has been transformed into a nanomolar integrin inhibitor using RGD and RGDF scaffolds to enhance its potency and selectivity for $\alpha v \beta 3$ integrins [134, 135]. New variants were also generated from a plasmid-borne gene cluster engineered to incorporate bulky non-canonical amino acids [136].

For comprehensive review covering all literature related to microcin J25, from its discovery and biosynthetic gene cluster to the characterization of its three-dimensional lasso structure, the readers are referred to [137].

Lassomycin is an excellent example of a lasso peptide that undergoes additional PTMs, specifically the addition of a C-terminal methyl ester to the mature peptide [17]. This modification plays a crucial role in the peptide's biological activity and stability. *LasF*, the final gene product in the lassomycin biosynthetic cluster, is a putative O-methyltransferase responsible for adding the C-terminal methyl ester to the mature peptide [17]. Svceucin is designed by heterologous expression from *Streptomyces coelicolor* [19]. Interestingly, it was demonstrated that macro-lactam SviC synthetase, though structurally different from McjC, can replace it in MccJ25 maturation with comparable efficiency [19]. Ubonodin is a 28-mer lasso peptide encoded in the genome of *Burkholderia ubonensis* [37]. Ubonodin uniquely has a large 18-residue loop, which includes a prominent turn from His15 to Trp19 [37]. A new variant of ubonodin has been identified with enhanced activity compared to wild-type ubonodin, exhibiting a submicromolar minimum inhibitory concentration (MIC) against the clinical isolate *Burkholderia cenocepacia*, a member of the *Burkholderia cepacia* complex (Bcc) [139].

With the incorporation of genome mining, the production of lasso peptides has expanded, enabling the discovery of new variants with potential applications. A new lasso peptide has been identified from *Burkholderia thailandensis* E264, named capistruin [77]. Capistruin

exhibits antibacterial activity against both Gram-positive and Gram-negative bacteria, including *Burkholderia caledonica*, *E. coli* 363, and *Pseudomonas aeruginosa* AT27853. Remarkably, capistruin outperforms microcin J25, which is inactive against *Burkholderia* and *Pseudomonas* strains [77]. This underscores the potential of genome mining for discovering potent lasso peptides. Other studies employed bio-orthogonal chemistry and heterologous expression to modify capistruin post-biosynthetically [140–142]. A crystal structure study of microcin J25 (MccJ25) and capistruin, revealed that both peptides bind to the bacterial RNAP within its secondary channel [143]. This binding blocks the folding of the trigger loop, a critical component for efficient RNAP catalysis, thereby inhibiting its enzymatic activity [143]. The study confirmed that microcin J25 (MccJ25) binds deeply within the RNAP secondary channel, likely interfering with nucleoside triphosphates (NTP) substrate binding and explaining its partial competitive inhibition mechanism [143]. In contrast, capistruin binds further from the RNAP active site, avoiding direct interference with NTP binding, which results in its partially noncompetitive inhibition mechanism [143].

The researchers observed that many lasso peptide clusters identified in their genome mining study, including the astexin-1 cluster, lack a dedicated ABC transporter, unlike those found in the MccJ25 and capistruin clusters [144]. This suggests that astexin-1 might use an existing ABC transporter in *E. coli*. This hypothesis is supported by the fact that MccJ25 can utilize the YojI transporter as an alternative to McjD [144]. This was also noted in the case of caulosegnins lasso peptides [93]. Interest in astexin remains high, with successful heterologous expressing of astexin-2 and -3 [35]. This research paves the way for engineering lasso peptides by revealing the roles of the *AtxE1* and *AtxE2* gene clusters. These clusters catalyze the reverse reaction of lasso peptide synthesis by cleaving the isopeptide bond formed during maturation [35,145]. Similarly, other studies demonstrated that sphingopyxin-IsoP, a POP-like serine protease, specifically targets threaded lasso peptides. Given that canonical serine proteases hydrolyze linear peptide substrates via an acyl-enzyme intermediate, their substrate specificity is partially determined by the complementarity between the enzyme's specificity pocket and the amino acid at the P1 position, which is the residue preceding the labile peptide bond [146,147]. Although it does not hydrolyze other lasso peptides, it does act on a variant of sphingopyxin I, indicating that the enzyme can accommodate subtle structural changes and likely recognizes various topology-conferring features [52].

Cloacaenodin has been confirmed to be recruiting a unique TonB-dependent transporter, CloU, to penetrate the outer membrane of susceptible bacteria, with transport across the inner membrane mediated by SbmA [148]. CloU is distinct from known lasso peptide transporters like FhuA and PupB and is primarily found in *Enterobacter* species, explaining cloacaenodin's activity spectrum [148]. Achromonodins I and II comprise Lys28 and Tyr29 as the upper and lower steric plugs, respectively [129]. Lys is relatively uncommon as a steric plug, as most lasso peptides use bulkier amino acids such as phenylalanine, tryptophan, tyrosine, or arginine [129].

7.3. Antiviral lasso peptides

Lasso peptides show promise as antiviral agents due to their unique structures and diverse mechanisms of action (Table 3).

They can act against viruses by (i) interacting with lipid membranes to disrupt viral envelopes, like siamycin-I [150–154] or forming pores that lead to viral inactivation or hinder the virus's ability to fuse with host cell membranes, such as NP-06 [149], (ii) inhibiting essential viral proteins, such as proteases, polymerases, and other enzymes critical for the viral life cycle, such as RP 71955 [42], or (iii) stimulating the host immune system.

Table 2

Summary of the antibacterial lasso peptides.

#	Lasso peptide		S-S bridges	Sequence	Target and mode of action	Ref
1	Microcin (MccJ25)	J25	0	GGAGHVPEYFVGIGT PISFYG	Gram negative bacteria. Inhibits bacterial transcription by binding to and blocking the nucleotide uptake channel of bacterial RNA polymerase (RNAP)	[45, 118-122]
2	Lariatins A and B		0	A: GSQLVYREWVGHSN VIKP B: GSQLVYREWVGHSN VIKGPP	Inhibit the growth of <i>Mycobacterium tuberculosis</i> . Their target is located within the cell wall of mycobacteria	[44]
3	Capistruin		0	GTPGFQTPDARVISR FGFN	Antibacterial (against Gram-positive and Gram-negative bacteria). bind to the bacterial RNAP.	[77]
4	Astexin-1		0	GLSQGVEPDIGQTYF EESRINQD	Antimicrobial activity against <i>C. crescentus</i>	[34]
5	Astexin-2 and 3		0	2: GLTQIQALDSVSGQF RDQLGL 3: GPTPMVGLDSVSGQ YWDQHAPLAD	Modest narrow spectrum antimicrobial activity	[35]
6	Streptomonicin		0	SLGSSPYNDILGYPALI VIYP	Antimicrobial activity (against various Gram-positive bacteria)	[48, 49]
7	Lassomycin		0	GLRRLFADQLVGRNI -CO ₂ CH ₃	Bactericidal against mycobacteria. Specifically targets ClpC1 ATPase, a crucial enzyme in mycobacteria	[17]
8	Sviceucin		2	CVWGGDCTDFLGCG TAWICV	Antimicrobial activity against Gram-positive bacteria and inhibited Fsr quorum sensing in <i>Enterococcus faecalis</i>	[19]
9	Sphaericin		0	GLPIGWWIERSGWY FPI	Antibacterial activity against <i>Micrococcus luteus</i>	[123]
10	Citrocin		0	GGVGKIIIFYGGVG RYG	Antimicrobial activity against enterohemorrhagic <i>E. coli</i> (EHEC) O157 TUV93–0. It binds to RNAP.	[99]
11	Specialicin		1	CLGVGSCVDFAGCGY AVVCFw	Antibacterial activity against <i>Micrococcus luteus</i> and the moderate anti-HIV activity against HIV-1 NL4-3	[124]

(continued on next page)

Table 2 (continued)

12	Ubonodin	0	GGDGSIAEYFNRP IHDWQIMDSGYG	potent antimicrobial activity against various pathogenic <i>Burkholderia</i> species, including those associated with lung infections in CF patients	[37]
13	Aborycin	2	CLGIGSCNDFAGCGY AVVCFW	Antibacterial activity against 13 <i>Staphylococcus aureus</i> strains, against <i>Enterococcus faecalis</i> ATCC 29212, against <i>Bacillus thuringiensis</i> , and against the poultry pathogen <i>Enterococcus gallinarum</i> 5F52C	[125]
14	Humidimycin	2	CLGIGSCDDFAGCGY AIVCFW		[126]
15	Arcumycin	2	CLPSGDCPDLGCGR AIWCI	Antibiotic activity against Gram-positive bacteria, including <i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , and <i>Micrococcus luteus</i> , also it enhances the activity of the fungal cell wall inhibitor caspofungin. Interferes with lipid II biosynthesis	[127]
16	Microcin Y (MccY)	0	GGRGHIAEYFSGPITQ VSFYG	antibacterial activity against some Gram-positive and Gram-negative bacteria, including some <i>Salmonella</i> and <i>Shigella</i> strains that are highly resistant to MccJ25	[128]
17	Triculamin and Alboverticillin		SKKSKPGDGIRKGV RG	Anti-mycobacterial activity against mycobacteria	[50]
18	Achromonodins 1 and 2	0[129]	1: GGGGPTPEYFLMPID PAWLQANLPNTGKYN 2: GSGGPIEYFYAPDP MGWKNPNVTKYG	Antimicrobial activity against <i>Achromobacter pulmonis</i> . Also, binds to and inhibits RNAP	

Red, isopeptide bond between N-terminal and either β-/γ-carboxy of Asp/Glu; green, modified C-terminal; NR, not reported; Ac, acetyl. [123–128].

7.4. Anticancer lasso peptides

Lasso peptides have gained attention for their potential anticancer properties in addition to their known antimicrobial activity. Their unique structure and diverse mechanisms of action make them promising candidates for the development of novel cancer therapies (Table 4). Sungsanpin is the first lasso peptide reported from a marine-derived microorganism [8]. Sungsanpin showed inhibitory activity in a cell invasion assay using the human non-small-cell lung cancer (NSCLC) cell line A549 [8]. Sungsanpin elevated the mRNA levels of tissue inhibitors of metalloproteinases (TIMP-1) and (TIMP-2) in a concentration-dependent manner, whereas the levels of matrix

metalloproteinases MMP-2 and MMP-9 remained unaffected [8]. Thus, reducing A549 cell invasiveness by upregulating TIMP-1 and TIMP-2, which inhibit MMPs [8]. Chaxapeptin [9] and sungsanpin [8] share a similar sequence and structure, both containing an eight-residue ring, a four-residue loop, and a three-residue tail. Interestingly, three out of the four differing residues are found in the tail (Ser-Trp-Leu vs. Asn-Phe-Phe, in positions 13–15). Despite this variation, their biological activity remains unaffected, with both peptides showing inhibitory effects on the invasion of A549 lung cancer cells [8,9] This study reinforces that the biological activity of lasso peptides is not linked to the tail region, but is instead associated with the macrolactam ring and the overall 3D structure of the peptide. Given that ulleungdin shows

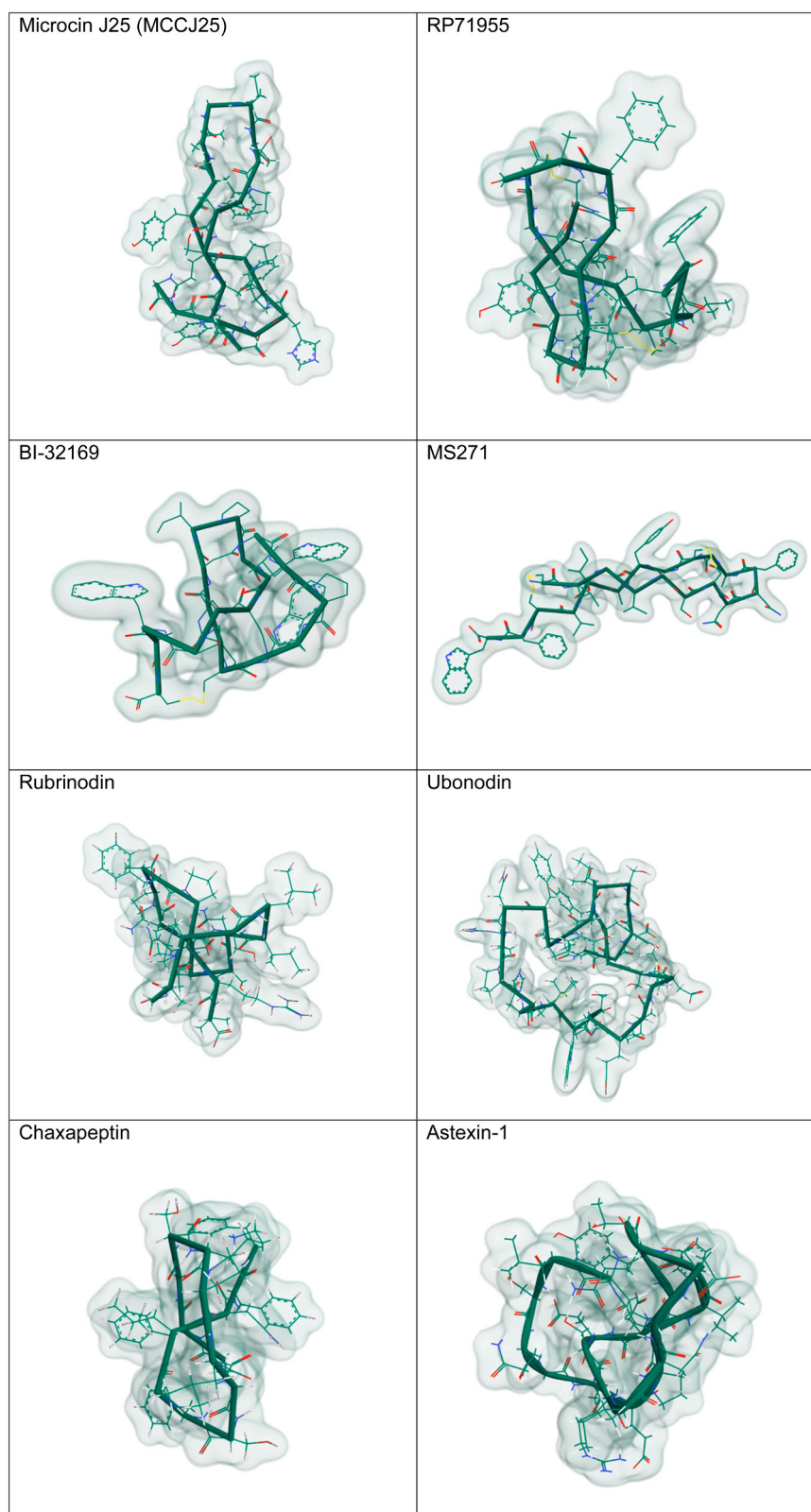


Fig. 5. 3D structures of selected lasso peptides. PDB files; 1Q71 [121], Microcin J25; 1RPB [42], RP71955; 3NJW [104], BI-32169; AF_AFP85078F1, MS271; Rubrinodin, 5OQZ [138]; Ubonodin, 6POR [37]; Chaxapeptin, 2N5C [9]; Astexin, 12LTI [34].

bioactivity comparable to sungsanpin and chaxapeptin, the authors suggest that the five residues common to these three peptides might be crucial for their biological activities [155].

Strikingly, non-threaded isomers of the lasso peptides sungsanpin

and ulleungdin were synthesized via SPPS [11]. Despite differences in the overall structure, these non-threaded isomers showed similar activity to the natural lasso peptides in inhibiting H1299 cancer cell migration [11]. Li's work further supported this observation by

Table 3
Summary of the antiviral lasso peptides.

#	Lasso peptide	S-S bridges	Sequence	Mode of action	Ref
1	NP-06	2	CLGVGSCNDFAGC GYAIVSFW	Antiviral (HIV-1). It forms pores in the viral lipid membranes	[149]
2	RP 71955	2	CLGIGSCNDFAGC GYAVVCFW	Antiviral (HIV-1). It inhibits replication enzymes	[42]
3	Siamycins II and II	2	I: CLGVGSCNDFAGC GYAIVCFW II: CLGIGSCNDFAGC GYAIVCFW	1. interacts with lipid II resulting in the activation of the lial stress response. 2. it inhibits PBP2 from incorporating disaccharide units into the expanding peptidoglycan network	[150, 151]

Red, isopeptide bond between N-terminal and either β -/ γ -carboxy of Asp/Glu; green, modified C-terminal; NR, not reported; Ac, acetyl.

synthesizing non-threaded sungsanpin analogues using SPPS, indicating that the lasso motif is not required for this type of activity [12]. These findings demonstrated that the lasso motif is not essential for inhibiting cancer cell migration [11].

A recent study indicates that ⁶⁴Cu-labeling methods are effective for synthesizing MccJ25 with an RGD motif-based PET probes [157]. The [⁶⁴Cu]Cu-MccJ25 variants are valuable tools for cancer molecular imaging, allowing for the visualization of integrin $\alpha\beta3$ -positive tumors with minimal background interference [157]. It is worth noting that MccJ25 has previously been converted into a nanomolar integrin inhibitor through the substitution of RGD and RGDF sequences [134,135].

7.5. Lasso peptides with no reported activity or inactivity

The following lasso peptides either showed no activity or have not been examined but were discovered during the development and investigation of various analogues with intriguing modifications and synthetic pathways (Table 5).

An intriguing, acetylated lasso peptide from *Streptomyces albus*, has been reported and named albusnodin, which features PTM via an acetyl group [159]. Through heterologous expression, the researchers claimed that acetylation might function as a resistance mechanism, protecting the producing cells from self-poisoning [159]. The production of albusnodin only occurs upon coexpression of a tailoring acetyltransferase enzyme, suggesting that acetylation may be an essential PTM required for the formation of the peptide [159].

Pseudomycoidin has been reported as a novel lasso peptide, encoded by *Bacillus pseudomycoides* DSM 12442. Pseudomycoidin has been produced *in vivo* from its ribosomally synthesized core part without the need for a leader protease [18]. This peptide uniquely undergoes macro-lactam formation directly by a macrolactam synthetase [18]. The researchers predicted that pseudomycoidin undergoes phosphorylation of a terminal residue—previously seen in other lasso peptides [15], followed by an unprecedented glycosylation modification [18]. This work demonstrates that a lasso peptide can be synthesized *in vivo* without the B1/B2 enzymes, which were previously thought to be essential for lasso peptide biosynthesis. Typically, the B1 protein recognizes the precursor leader sequence and recruits the B2 protease to cleave at the leader–core junction, generating an N-terminus used by the C protein to form a macrolactam ring with a Glu or Asp side chain in the core peptide. However, for pseudomycoidin, this requirement is bypassed by a cellular protease(s) that cleaves at the leader–core junction [18]. The authors explained that, since no cleavage occurred with PsmB1 or

PsmB2 used individually, the role of these proteins is likely performed by host proteins [18]. The study concludes that the C protein can independently convert the peptide into a lasso structure, as evidenced by the efficient synthesis of pseudomycoidin with only the PsmA core peptide and PsmC present [18]. The crystal structure of the B1 protein from the thermophilic actinobacterium *Thermobifida fusca* (TfuB1) has been reported in complex with the leader peptide (TfuA-Leader) [64]. The study highlights that the leader peptide binds to one edge of the β -sheet in TfuB1, forming an intermolecular β -sheet [64]. The crystal structure of the TfuB1-TfuA complex has provided definitive structural details of the B1 protein and its interaction with the leader peptide [64]. However, the researchers emphasized the need for further structural analysis of the B2 protein in complex with both the precursor peptide and the B1 protein to elucidate the cleavage mechanism and the detailed interactions between the precursor peptide and B1 protein [64].

The researchers discovered a kinase within paeninodin’s biosynthetic cluster and identified it as part of a new class of lasso peptide-tailoring kinases [15]. While enzymes that phosphorylate internal Ser residues are common, the enzyme identified in their study uniquely phosphorylates a C-terminal Ser residue [15].

In Cellulonodin-2, the researchers found that protein l-isoaspartyl methyltransferase (PIMT)-mediated methylation of isoAsp leads to succinimide formation, which is then hydrolyzed to isoAsp and Asp [161]. This modification occurs at an Asp residue in the lasso peptide ring [161]. As demonstrated with lassomycin [17], this study also revealed that O-methyltransferases act exclusively on mature lasso peptides, unlike other lasso peptide PTMs, which occur on precursors [161]. Michaelis–Menten kinetic measurements for aspartimide formation and hydrolysis in the lasso peptide lihuonodin, along with the kinetics of the entire reaction network, have also been reported [162].

8. Conclusion and future perspectives

This critical review highlights the remarkable diversity and biological potential of lasso peptides, a unique class of natural molecules characterized by their intriguing structural properties and extensive PTMs. The exploration of these peptides has revealed that their macro-lactam ring, particularly the lasso motif, plays a pivotal role in maintaining structural integrity and biological function. Notably, the ability to redesign lasso peptides through amino acid substitutions, especially within the ring and tail regions, opens avenues for the development of novel analogues with enhanced biological activities.

Emerging findings indicate that while the lasso structure contributes

Table 4
Summary of the anticancer lasso peptides.

#	Lasso peptide	S-S bridges	Sequence	Mode of action	Ref
1	Sungsanpin	0	GFGSKPIDSFGLSWL	Inhibit the invasiveness in NSCLC. It upregulates TIMP-1 and TIMP-2, which inhibit MMPs	[8]
2	Chaxapeptin	0	GFGSKPLDSFGLNFF	Weak antibacterial effect against Gram-positive strains such as <i>Staphylococcus aureus</i> and <i>Bacillus subtilis</i> . Inhibit invasiveness IN human lung cancer cell line A549	[9]
3	Bicyclic peptide 5	0	GFESKPIDSFGLSWL	Inhibit invasiveness IN human lung cancer cell line A549	[87]
4	Ulleungdin	0	GFIGWGKDIFGHYGG	Inhibit activities against cancer cell invasion and migration	[155]
5	Felipeptins A1 and A2	1	GSRGWGFEPGVRCLIW CD GGGRGYEYNKQCLIF C	Accelerates cancer cell proliferation, thereby significantly increases sensitivity to the anticancer drug doxorubicin	[39]
6	Cloacaenodin	0	GHSVDRIPEYFGPPGLP GPVLFYS	antimicrobial activity against species related to the <i>E. cloacae</i> complex, including those implicated in nosocomial infections and clinical isolates of carbapenem-resistant Enterobacterales. It uses a unique TonB-dependent transporter, CloU, to penetrate the outer membrane of susceptible bacteria, with transport across the inner membrane mediated by SbmA	[148, 156]

Red, isopeptide bond between N-terminal and either β -/ γ -carboxy of Asp/Glu; green, modified C-terminal; NR, not reported; Ac, acetyl; TIMP, Tissue inhibitor of metalloproteinase. [156].

Table 5

Summary of the discussed lasso peptides with no relevant/reported activity.

#	Lasso peptide	S-S bridges	Sequence	Biological activity	Ref
1	Caulosegnins I–III	0	I: GAFVGQPEAVNPLGREI QG II: GTLTPGLPEDFLPGHYM PG III: GALVGLLLEDITVARYDP M	NR	[93]
2	Xanthomonins I–III	0	I: GGPLAGEEIGGFNVPG II: GGPLAGEEMGGITT III: GGAGAGEVNGMSP	NR	[58]
3	Caulonodin IV–VII	0	IV: SFDVGTIKEGLVSQYYFA V: SIGDSGLRESMSSQTYW P VI: AGTGVLLPETNQIKRYD PA VII: SGIGDVFPENMVRRED	NR	[51]
4	Sphingopyxin and II	0	I: GPGGITGDVGLGENNFG II: GEALIDQDVGGGRQQFL TG	Inactive	[52]
5	Paeninodin	0	AGPGTSTPDAFQDPDDE DVHYDS	NR	[52]
6	Subterisin	0	PPGDRIEFGVLAQLPG	NR	[158]
7	Acinetodin	0	GGKGPIFETWVTEGNY G	Inactive	[90]
8	Klebsidin	0	GSDGPHEFFNPNGVMH YG	Inactive	[90]
9	Cattlecin	0	SYHWGDYHDWHHWY GW WDD	Inactive	[53]
10	Benenodin-1	0	GVGFGRPDSILTQEQAK PM	NR	[96]
11	Albusnodin	0	GQGGGQSEDK(Ac)RRA YNC	NR	[159]
12	Brevunsin	0	DGMGEEFI EGLVRDSL PPAG	Inactive	[54]
13	Fuscanodin	0	WYTAEWGLELIFVPRFI	NR	[55]
14	Mycetohabin-15	0	GGSGQYREAGVGRFL	Inactive	[67]
15	Mycetohabin-16	0	GGSGKYKEAGVGRFLD	Inactive	[67]
16	Burhizin-23	0	GGAGQYKEVEAGRWS RIDSDE	Inactive	[67]
17	Pseudomycoidin	0	AGPGKRLVDQVFEDE QCALHHS	Inactive	[18]
18	Pandonodin	1	GVLGNDAGEGITLLPLCFK PICIPTLPPLTGGA	NR	[36]
19	Huascoptin	0	GYGNAWDSKNGLF	Inactive	[38]
20	Rubrinodin	0	GTIDPQNSEEHVLSRR LEN	Inactive	[95]
21	Mycetolassin-15 and 18	0	15: GGSGQYREAGVGRFF 18: GGTGQYKEVGIGRFYD GA		[142]
22	Koreensin	0	GPKGDFPDVGDGRILAG	No cell adhesion inhibitory activity	[160]
23	Koreensin-RGD	0	GPRGDFPDVGDGRILAG	No cell adhesion inhibitory activity	[160]

Red, isopeptide bond between N-terminal and either β -/ γ -carboxy of Asp/Glu; green, modified C-terminal; NR, not reported; Ac, acetyl. [158,160].

to stability, specific key residues are essential for imparting biological effects. Genome mining offers exciting prospects for engineering these peptides, allowing for the production of new derivatives that retain their conserved 3D structures while potentially enhancing their pharmacological properties. Recent advancements, such as the introduction of non-canonical amino acids (ncAAs) [136] and innovative software tools for computational prediction, underscore the potential for high-throughput exploration and design of lasso peptides [20–22].

The future of SPPS looks particularly promising. Recent advancements in the development of a cryptand-imidazolium complex have enabled the creation of a multi-linker support for synthesizing the lasso peptide BI-32169 [88], which can be effectively utilized in SPPS. However, innovative synthetic technologies are essential to fully realize this potential. In our laboratory, we have redefined SPPS to enable the synthesis of first-in-class peptide structures [89]. We are committed to synthetically delivering lasso peptides, building on the insights gained from the previously reported cryptand-imidazolium complex, which was explored in solution phase. This approach will significantly expand the scope of lasso peptide libraries.

Looking forward, continued research into the diverse PTMs associated with lasso peptides, including citrullination [40], phosphorylation [15], C-terminal methylation [16,17], and glycosylation [18], will provide deeper insights into their functional roles and therapeutic applications. The integration of bioinformatics and machine learning will further facilitate the discovery and optimization of lasso peptides, paving the way for new antibiotic development and therapeutic interventions [163]. Future studies should aim to elucidate the precise mechanisms by which these peptides exert their biological effects, potentially leading to breakthroughs in drug design and development strategies.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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