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Nonribosomal Peptide Synthesis

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Abstract

Nonribosomal peptides (NRPs) are a type of secondary metabolite with a wide range of pharmacological and biological activities including cytostatics, immunosuppressants or anticancer agents, antibiotics, pigments, siderophores, toxins. NRPs, unlike other proteins, are synthesized on huge nonribosomal peptide synthetase (NRPS) enzyme complexes that are not dependent on ribosomal machinery. Bacteria and fungi are the most common NRPs producers. Furthermore, the presence of these peptides has been confirmed in marine microbes. Nowadays, many of these peptides are used in the treatments of inflammatory, cancer, neurodegenerative disorders, and infectious disease for the development of new therapeutic agents. The structure, function, and synthesis of NRPs, as well as producer microorganisms and their several application areas, are covered in this chapter.

Keywords: biological activity, nonribosomal peptide, producer microorganisms, secondary metabolite, synthesis, structure

1. Introduction

Bioprocesses, which are consisted of a series of enzymatically catalyzed biochemical reactions in all living things, are necessary for survival. They have a high potential in terms of material synthesis, which has recently been performed by chemical techniques [1]. Furthermore, the advancement of heterologous production systems and genetic engineering techniques has resulted in pioneering initiatives to manufacture usable biomaterials [2]. These advancements also enabled the successful generation of primary and secondary metabolic pathway products in physiologically and genetically well-defined hosts, such as *Escherichia coli* and *Saccharomyces cerevisiae*, by precise manipulation of the related genes. In particular, heterologous molecular hosts have been used to successfully synthesize structurally varied secondary metabolites showing unique pharmacological action [1–3]. Nonribosomal peptides (NRPs) obtained by the most extensive, appealing, and useful actively-studied bioprocesses are included among these metabolites, which are important in the discovery and development of drugs and therapeutic reagents [1, 4].

NRPs are secondary metabolites that are synthesized outside of the ribosomal machinery and have a variety of properties such as cytostatics, immunosuppressants or anticancer agents, antibiotics, pigments, siderophores, toxins [3, 5, 6]. NRPs are typically produced by marine microorganisms and invertebrates, as well as

soil-inhabiting microorganisms [5, 7, 8]. The majority of natural products produced by sponges, bryozoans, mollusks, and tunicates are members of the NRP or mixed polyketide–NRP families. Several of NRPs are being used in the development of new medicines for inflammatory, cancer, neurological diseases, and infectious disease nowadays [7].

Non-ribosomal peptide synthetases (NRPSs) enzymes are poly-functional mega-synthases that biosynthesize NRPs [7, 9, 10]. NRPSs, multi-modular enzyme or enzyme complexes from common bacteria, less common eukarya, and rare archaea, are capable of producing a wide range of natural pharmaceutical products (Bacitracin, antibiotics for skin infections; Bleomycin antitumor; Cyclosporin, antifungal, and immunosuppressive drugs; Daptomycin, antibiotics) [5, 7, 11]. NRPSs use both proteinogenic and nonproteinogenic amino acids (not encoded by DNA) as building blocks for the growing peptide chain [1, 7, 11, 12]. They catalyze multiple biosynthetic processes, each of which is responsible for particular one amino acid elongation on the growing peptide chain [10]. This chapter looks at the structure, function, and synthesis of NRPs, as well as producer microorganisms and their various applications.

2. Synthesis, structure, and function of nonribosomal peptide (NRP)

NRPSs are responsible for nonribosomal peptide (NRP) synthesis. These are large multi-enzyme complexes that are modularly organized and serve as biosynthetic machinery and templates [5, 11–14]. For example, a single NRPS of 1.6 MDa synthesizes the Cyclosporine A (7). In fungal systems, such as in the case of cyclosporine A (7), a single NRPS synthesizes peptides, whereas bacteria frequently use numerous NRPSs with genes grouped in an operon. NRPSs have a modular structure [14, 15].

In a genome mining research of 2699 genomes, Wang et al. found that more than half of the NRPS enzymes were non-modular NRPS enzymes [16]. Nonmodular NRPS enzymes can be found in siderophore biosynthesis pathways, such as EntE and VibH in enterobactin and VibE in vibriobactin, or as a standalone peptidyl carrier protein, such as BlmI from the bleomycin gene cluster. NRPS enzymes are found frequently in bacteria, less frequently in eukaryotes, and infrequently in archaea. Actinobacteria, Cyanobacteria, Firmicutes, and Proteobacteria were the phyla with the greatest number of these enzymes in the bacterial domain. There was a correlation between genome size and the number of NRPS clusters [5, 17].

A module is a part of the NRPS polypeptide chain that is in charge of integrating one amino acid into the final product. Modules can further be separated into domains (**Figure 1**), which represent enzyme units that catalyze distinct steps of NRP synthesis. On the protein level, domains are defined by distinctive, greatly conserved order of patterns known as "core motifs." In certain instances, biochemical and structural data were used to confirm the involvement of greatly conserved residues in domain function (**Table 1**) [14].

There are three domains in a module. These are 1) the adenylation (A) domain, 2) the peptidyl carrier protein (PCP) or thiolation (T) domain, and 3) the condensation (C) domain, all of which are responsible for the synthesis of NRPs. A module may include additional tailoring or altering domains incorporating epimerization (E), methylation and oxidation domains or a heterocyclization (Cy) domain in place of a C-domain. Finally, most NRPS termination modules have a TE-domain, which is in charge of releasing linear, cyclic, or branching cyclic peptides [5, 9–11, 15, 18–21].
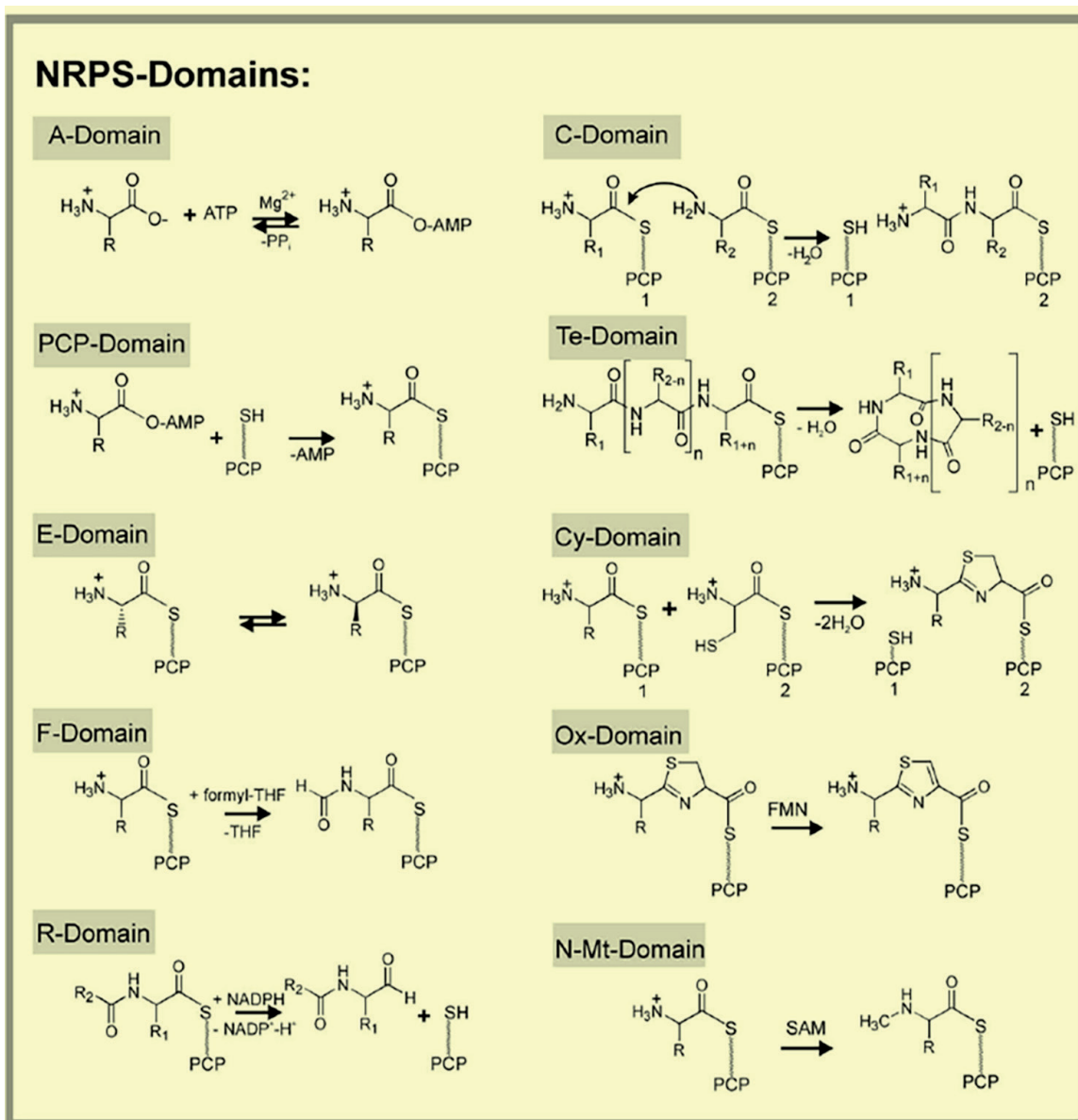
Figure 1.
Catalyzed reactions by various NRPS-domains [14].

The order of the modules is frequently aligned with the sequences of the resulting peptides. NRP synthesis begins at the N-terminus and ends at the C-terminus, yielding peptides that are typically 3–15 amino acids long. The released peptides contain amino acids, that is, imino acids or ornithine and their structures are linear, cyclic-macrocyclic, branched-cyclic, branched-macrocyclic, dimers or trimers of identical structural elements [5].

The A-domain is responsible for the first step in biosynthesis, which involves recognizing and activating the amino acid substrate via adenylation with Mg-ATP, resulting in an aminoacyl adenylated intermediate. Around 550 amino acids make up domain A. It has 10 amino acid residues that serve as NRPS enzyme "codons" and are essential for substrate specificity. The D and L forms of the 20 amino acids used in ribosomal protein synthesis, as well as non-proteinogenic amino acids like imino acids, ornithine, and hydroxy acids like β-butyric acids and α-aminoadipic, are substrates recognized by the A-domain. The PCP-domain, which consists of about 80 amino acids and covalently attaches the activated amino acid to their cofactor 4′-phosphopantetheine (PP)

A-domains		PCP-domains	
A1	L(TS)YxEL	T	LGG(DH)SL
A2	LKAGxAYL(VL)P(LI)D		
A3	LAYxxYTSG(ST)TGxPKG		
A4	FDxS		
A5	NxYGPTE		
A6	GELxJGx(VL)ARGYL		
A7	Y(RK)TGDL		
A8	GRxPxQVKIRGxRIELGEIE		
A9	LPxYM(IV)P		
A10	NGK(VL)DR		

C-domains		Te-domains	
C1	SxAQxR(LM)(WY)xL	Te	GxSxG
C2	RHExLRTxF		
C3	MHHxISDG(WV)S		
C4	YxD(FY)AVW		
C5	(IV)GxFVNT(QL)(CA)xR		
C6	HN)QD(YD)PFE		
C7	RDxSRNPL		

E-domains		Cy-domains	
E1	PIQxWF	Cy1	FPL(TS)xxQxAYxxGR
E2	HHxISDG(WV)S	Cy2	RHx(IM)L(PAL)x(ND)GxQ
E3	DxLLxAxG	C3	D(NLI)xDxxS
E4	EGHGRE	Cy3	LPxxPxLPLxxxP
E5	RTVGWFTxxYP(YV)PFE	Cy4	(TS)(PA)3x(LAF)6x(IVT)LxxW
E6	PxxGxGYG	Cy5	(GA)DFTxLxLL
E7	FNYLG(QR)	Cy6	PVVFTSxL
		Cy7	(ST)(QR)TPQVx(LI)D13xWD

Ox-domains		N-Mt-domains	
Ox1	KYxYxSxGxxY(PG)VQ	M1	VL(DE)xGxGxG
Ox2	GxxxG(LV)xxGxYYY(HD)P	M2	NELSxYRYxAV
Ox3	IxxxYG	M3	VExSxARQxGxLD

R-domains	
R1	V(L)(L)TG(A)TG(F)(L)GxxLL
R2	Vx(L)(L)VR(A)
R3	GPL(G)x(P)x(L)GL
R4	V(Y)PYxYLxx(P)NVxxT
R5	GYxxSKW(A)(A)E
R6	R(P)G
R7	YxxxxG(LF)LxxP

Table 1.
NPRS-domains' core-motifs [14].

arm via a thioester bond, completes the second step. And also, the active substrate and elongation intermediates are transferred to the C-domain via this domain. In the last step, C-domain, which contains approximately 450 amino acids, catalyzes the formation of peptide bonds between the carboxyl group of the incipiently synthesized peptide and the amino acid transported by the side module [5, 22]. Furthermore, this domain allows the expanding chain to be translocated to the next module. Following this step, the linear intermediate peptide is liberated in bacteria via internal cyclization or hydrolysis with the help of the Thioesterase (TE) domain. On the other hand, it

appears less commonly in fungi's NRPSs. Fungi use a variety of ways to release chains. The first is a thioesterase NADP(H)-dependent reductase domain (R), which catalyzes NADPH reduction to create an aldehyde and the second is a terminal C domain, which catalyzes release by forming intermolecular or intramolecular amide bonds. By N-, C-, and O-methylation, halogenation, acylation, hydroxylation, glycosylation, or heterocyclic ring formation, the primary product of this synthesis can be changed post-synthetically to reach its mature form by additional tailoring enzymes that are not part of the NRPS. The structural diversity of NRPs is formed in part by these enzymes and their reactions [5].

Because of their extensive multidomain organization, NRPS genes are easier to identify using recent genome mining technologies, and they are also relatively easy to detect. Secondary metabolites production genes are frequently found in bacterial and fungal gene clusters. The clusters' core is thought to be NRPS genes. Nevertheless, they are linked to genes involved in building blocks synthesis, product ornamentation, self-resistance, and peptide export. For the purpose of analyzing and in silico exploration of NRPS pathways, advanced genome sequencing techniques have made genome mining methodologies available, which are assisted by a variety of bioinformatics tools, such as AntiSMASH, PRISM, and SMURF [23].

Nowadays, known NRP structures are divided into various categories, each with its own structural characteristics. Lipocyclopeptides with varied linkage patterns, such as fengycin, iturin, surfactin, and head-to-tail-cyclized peptides of varying ring sizes, such as cyclosporine, gramicidin S, maybe the largest group. There are also a lot of linear peptide configurations. They include tripeptides (such as sevadicin and bialaphos) as well as 20-mer peptides (e.g., alamethicin, peptaibols). The current highest size limit for NRPs is syringopeptin 25A, which has 25 amino acids (syringopeptin 25A). Tailoring enzymes modify the structure of some NRPs. The most structurally complicated molecules are probably bleomycins, ergopeptides, glycopeptide antibiotics, and β-lactams [23].

Figure 2 shows some NRPs with diverse structures and a wide spectrum of activities. ACV-tripeptide (6), for example, is a precursor to antibiotics of the penicillin and cephalosporin families. Gramicidin S (4), tyrocidine A (1), and vancomycin (5) are three other antibiotic-active substances. Cyclosporin A (7), an immunosuppressive drug, is used in the post-transplantation care of patients. Cancer is treated with cytostatic agents, such as bleomycin A2 (8) and epothilone (9). Enterobactin (10), bacillibactin (11), mixochelin A (12), yersiniabactin (13), and vibriobactin (14) are examples of iron chelating agents. These compounds, known as siderophores, are created in iron-deficient environments to provide bacteria with an iron source. **Figure 2** also depicts the structures of pigments like indigodin (15), toxins like thaxtomin A (17), and peptides with uncertain functions like anabaenopeptilide 90-A (18) [14].

NRPs have a number of structural characteristics that distinguish them from ribosomal peptides. For example, non-proteinogenic amino acids, such as ornithine in 1, 2, and 4, hydroxyphenyl or dihydroxyphenyl-glycine in 5 and (4R)-4-[(E)-2-butenyl]-4-methyl-L. -threonine (Bmt) in 7, are included. Furthermore, the structures are frequently macrocyclic (1), branched macrocyclic (2), or dimers of two (4) or trimers of three (10, 11) structural components. Smaller heterocyclic rings, such as thiazole in 9, thiazoline in 13, or oxazoline in 14, are common structural properties of nonribosomal peptides. In addition, fatty acids (3), glycosylations (5), N-methylations (7), and N-formylations (18) may also be present, as well as the addition of propionate units (8) or acetate [14].
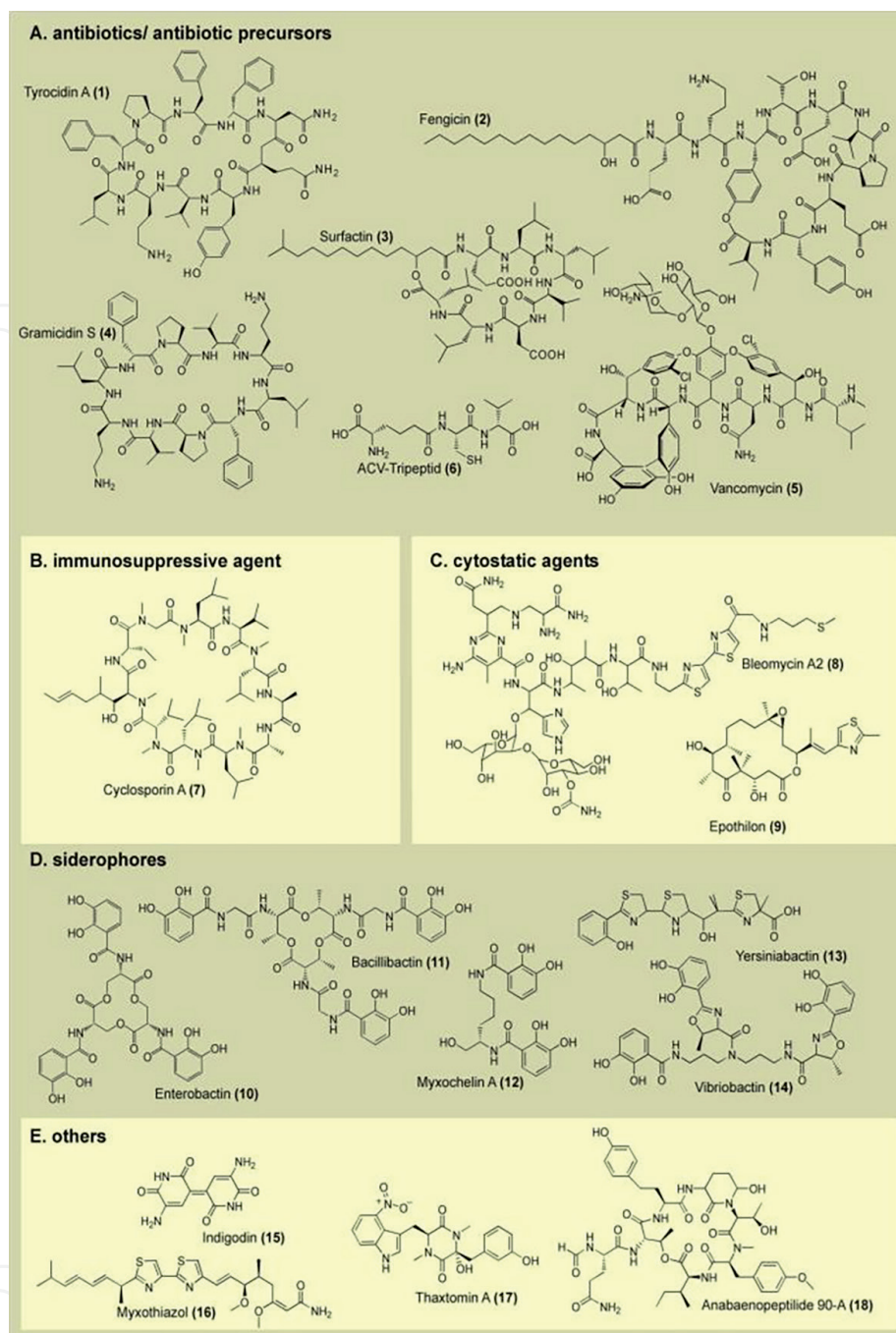
Figure 2.
Some NRPs with structural diversity [14].

3. Overview of producer microorganisms for NRP

NRPs are typically produced by marine microorganisms, soil-inhabiting micro-organisms, including *Actinomycetes*, *Bacilli,* and eukaryotic filamentous fungus, and invertebrates, such as sponges, bryozoans, mollusks, and tunicates [5, 7, 11, 13, 24]. Many pharmacologically active NRPs have been effectively generated in heterologous hosts, such as *Bacillus subtilis*, *Escherichia coli*, *Saccharomyces cerevisiae*, and *Streptomyces* sp. [2]. Bacteria and fungi are the primary producers of NRPS-based metabolites. Except for bacteria and fungus, NRPS Ebony from *Drosophila melanogaster* ("fruit fly") and nemamide synthetase from the worm *Caenorhabditis elegans* have been confirmed. The distribution and occurrence of NRPS pathways and

products have been discovered, thanks to screening efforts and genome sequencing projects followed by bioinformatics research. NRPS enzymes are found frequently in bacteria, less frequently in eukaryotes, and infrequently in archaea. The phylum Actinobacteria (*Mycobacterium*, *Streptomyces*), Firmicutes (*Bacillus*, *Staphylococcus*, and *Streptococcus*), and the alpha-/beta-/gama-Proteobacteria classes (*Burkholderia*, *Escherichia*, *Erwinia*, *Photorhabdus*, *Pseudomonas*, *Salmonella*, *Serratia*, *Vibrio*, and *Yersinia*) are the most important contributors among bacteria. Nonetheless, in recent years, the phylum Cyanobacteria (*Microcystis*, *Planktothrix*, *Anabaena*, *Oscillatoria*, and *Nostoc*) and the teta-Proteobacteria (*Myxobacterium*) class have received greater attention [5, 22, 23]. NPRS genes are found predominantly in the Ascomycota (*Tolypocladium*, *Fusarium*, *Penicillium*, *Acremonium*, *Claviceps*, and *Trichoderma*) and marginally in the Basidiomycota (*Ustilago*) phylum. NRPS biosynthesis investigations in fungus are less investigated than in bacteria due to greater genome sizes, the existence of scattered introns in gene clusters, and a less established molecular biology toolbox [23].

4. Application areas of NRPs

Novel peptide products' biological functions are strictly associated with their chemical structure, which is constrained by a peptide sequence that ensures specific interaction with a specific molecular target. Chemical alterations, such as the incorporation of fatty acid chains, D-amino acids, glycosylated amino acids, and heterocyclic rings, as well as cyclization or oxidative cross-linking of side chains, add a lot to these unique interactions. Bacitracin, fengycin, pristinamycin, surfactin, tyrocidine, and vancomycin are examples of novel peptides with antibacterial and antifungal properties [25].

When the ribosomal code was deciphered in the 1960s, Tatum and coworkers discovered that ribosomes had no effect on cell-based tyrocidine production [23, 26]. The first NRPs agent is tyrocidine, a cyclic decapeptide that is biosynthesized outside of the *Bacillus brevis* ribosome. Researchers discovered that ribosome targeting antibiotics had no effect on tyrocidine production. They also discovered that *B. brevis* can synthesize gramicidin S, a cyclic decapeptide, without the use of tRNA molecules or aminoacyl-tRNA synthetases [13, 27]. Nobel Prize Laureate Fritz Lipmann and Søren Laland contributed to present essential biochemical activity insights into NRPSs, including specific ATP-dependent activation of amino acids, thioester-mediated 4′-phosphopantetheine (Ppant) binding of activated amino acids, and the directionality of the peptide synthesis and have given acceleration to the production of NRPS-based metabolites synthesized by a mechanism distinct from protein synthesis. The NRPs and NRPSs were discovered as a result of these findings associated with the synthesis of tyrocidine and gramicidin S peptides. Surprisingly, the majority of studies investigating nonribosomal NRPS-based metabolites have focused on antibacterial and antifungal action [23]. NRPS-based metabolites with antimalarial, antimicrobial, antiparasitic, antiviral, animal growth promoters, cytostatic, immunosuppressive, and natural insecticides properties are currently available on the market, and several are being studied in clinical research [28]. **Table 2** presents a summary of commercialized NRPs-based medications with antibacterial activity.

As demonstrated in **Table 2**, systemic and topical antibacterials are the most often used NRPs-based drugs, accounting for billions of dollars in the chemical and pharmaceutical industry sales. **Table 3** lists their other applications, which include

Compound	Biosynthetic class of agent	Source	Disease/Molecular target
Bacitracin	Cyclic peptide	*Bacillus subtilis*	Antibiotic/dephosphorylation of C55-isoprenyl pyrophosphate
Bleomycin	Hybrid peptide	*Streptomyces verticillus*	Antibiotic/inhibition of DNA synthesis
Capreomycin	Cyclicpeptide	*Streptomyces capreolus*	Antibiotic/protein synthesis inhibitor
Carbapenems	Synthetic thienamycin	*Streptomyces cattleya*	Antibacterial (multidrug resistant)/bacterial cell-wall biosynthesis (peptidoglycan;β-lactamase inhibition)
Cephalosporin	β-lactam	*Acremonium chrysogenum*	Antibiotic/Alters bacterial outer membrane
Chloramphenicol	Synthetic;further derivatives: thiamphenicol [c], florfenicol	*Streptomyces venezuelae*	Antibacterial/inhibition of ribosomal protein synthesis
Colistin (Polymyxin E)	—	*Paenibacillus polymyxa var. colistinus*	Antibacterial/binding to lipopolysaccharide (outer membrane), interaction with the cytoplasmic membrane
Dalbavancin	Semisynthetic teicoplanin derivative	—	Antibacterial (Gram-positive)/membrane anchoring; disruption of cell membrane and inhibition of bacterial cell wall biosynthesis
Daptomycin	Lipopeptide	*Streptomyces roseosporus*	Antibiotic (Gram-positive)/disrupts the cell membrane
Gramicidin	Linear pentadecapeptide	*Bacillus brevis*	Antibiotic/ion-channel formation, increasing the permeability of the membrane
Lincomycin	—	*Streptomyces lincolnensis*	Antibacterial (patients allergic to penicillin) inhibition of the ribosomal protein synthesis (50S-subunit, dissociation of peptidyl-tRNA from the ribosome)
Monobactams	—	*Chromobacterium violaceum*	Antibacterial (Gram-negative)/bacterial cell-wall biosynthesis
Oritavancin	—	Semi synthetic	Antibiotic/disrupts the cell membrane
Polymyxin B	Polypeptides	*Bacillus polymxya*	Antibacterial (Gram-negative)/binding to lipopolysaccharide (outer membrane), interaction with cytoplasmic membrane
Pristinamycin	Depsipeptide	*Streptomyces pristinaespiralis*	Antibacterial (Gram-positive)/ribosomal biosynthesis (50S-subunit, peptidyl transfer, and elongation of protein synthesis)
Teicoplanin	Glycopeptide	*Actinoplanes teichomyceticus*	Antibiotic/inhibit cell wall synthesis
Telavancin	—	*Amycolatopsis orientalis*	Antibacterial (Gram-positive) disruption of cell membrane and inhibition of bacterial cell-wall biosynthesis

Compound	Biosynthetic class of agent	Source	Disease/Molecular target
Tyrothricin	—	*Bacillus brevis*	Antibacterial (Gram-positive)/ disruption of cell membrane
Vancomycin	Glycopeptide	*Amycolatopsis orientalis*	Antibiotic/inhibit cell wall synthesis
Virginiamycin	—	*Streptomyces virginiae*	Antibacterial/ribosomal biosynthesis (50S-subunit, peptidyl transfer, and elongation of protein synthesis)

Table 2.
Overview of NRPs-based drugs [7, 23].

Agent	Origin	Properties and area of application
Actinomycin D (Dactinomycin)	*Actinomyces antibioticus*, *Streptomyces chrysomallus*	Antitumor/DNA intercalator, inhibition of transcription
Bialaphos	*Streptomyces hygroscopicus*, *Streptomyces viridochromogenes*	Herbicide/tripeptide prodrug, inhibitor of glutamine synthetase
Bleomycin A2, B2	*Streptomyces verticillus*	Antitumor/metal-dependent oxidative cleavage of DNA in presence of molecular oxygen
Capreomycin	*Streptomyces capreolus*	Antituberculous/ inhibition of the ribosomal protein synthesis (16S and 23S-rRNA)
Carfilzomib	Synthetic derivative of epoxomycin (*Actinomyces* sp.)	Anticancer/proteasome inhibitor
Caspofungin	*Glarea lozoyensis*, semisynthetic from pneumocandin; further derivatives: micafungin/ anidulafungin	Antifungal (candidiasis, aspergillosis) fungal cell-wall integrity ((1-3)-β-D-glucan synthase)
Cyclosporine A	*Tolypocladium inflatum*	Immunosuppressant/cyclophilin binding, inhibition of IL-2 expression (inhibition of T-cell activation)
Emodepside	*Mycelia sterilia* (F); semisynthetic from PF1022A	Anthelmintic/Slo-1 receptor (K+ channel)
Enduracidin (Enramycin)	*Streptomyces fungicidicus*	Antibacterial, food additive/inhibition of MurG (essential for cell-wall biosynthesis in Gram positive bacteria), inhibition of the transglycosylation step of peptidoglycan biosynthesis
Enniatins (fusafungine)	*Fusarium lateritium*, *Fusarium scirpi*, *Fusarium* sp.	Antibacterial (topical), antifungal, anti-inflammatory/ ionophore (NH4⁺) membrane depolarization
Ergometrine (ergonovine)	*Claviceps purpurea*	Obstetrics/interaction with a-adrenergic, dopaminergic and serotonin receptors
Ergotamine	*Claviceps purpurea*	Migraine vasoconstrictive (5-HT1B receptor, but also dopamine and noradrenaline receptors)
Romidepsin	*Chromobacterium violaceum*	Antitumor/histone deacetylase inhibitor (inducing apoptosis)
Trabectedin	Bacterial symbiont of *Ecteinascidia turbinata* (sea squirt)	Antitumor (antiproliferative, treatment of soft tissue sarcoma) DNA binder, blocks binding of transcription factors

Table 3.
Marketed-NRPs agents [23].

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anticancer agents, antifungals, animal feed additives, immunosuppressants (cyclosporine), obstetrics (ergometrine), and pain management (ergotamine).

In the medical field, NRP-based marketed drugs, such as Cyclosporin A and Bleomycin A2, have high selling prices. The cost of these medicines is $107 for 25 mg of Cyclosporine A (98% purity) obtained from *T. inflatum* and $847 for 20 mg of Bleomycin A2 (70% purity) isolated from *S. verticillus*, according to Sigma Chemical Company [5].

The 70% discovery of NRPs with antibacterial, antiviral, cytostatic, immunosuppressive, antimalarial, antiparasitic, animal growth promoters, and natural insecticides activity is mostly attributed to marine organisms [13]. NRPs obtained from marine organisms (sponges, tunicates, and their associated phyla, such as Acidobacteria, Actinobacteria, Bacteriodetes, Chloroflexi, Cyanobacteria, Nitrospira, Planctomycetes, Poribacteria, Proteobacteria, Verrucomicrobia, and Archaea) have excellent binding properties, low off-target toxicity, and high stability and these properties make them a promising molecule for the development of new therapeutics pharmacologically active in many clinical searches. **Table 4** shows the chemical structure and source of various NRPs isolated from marine sponges and tunicates.

NRPs agents	Chemical class	Origin	Target
Miraziridine A	Linear pentapeptide	*Theonella* aff. *mirabilis*	Cancer/inhibit protease cathepsin B
Haligramides A-B	Cyclic hexapeptides	*Haliclona nigra*	Cancer/A-549 (lung), HCT-15 (colon), SF-539 (CNS), SNB-19 (CNS)
Prepatellamide A	Cyclic peptide	*Lissoclinum patella*	Cancer/P388 murine leukemia cell lines
Tamandarins A-B	Depsipeptides	*Didemnid ascidian*	Cancer/pancreatic carcinoma BX-PC3, prostatic cancer DU-145, head and neck carcinoma UMSCC10b
Microsclerodermins F–I	Cyclic peptides	*Microscleroderma* sp.	Cancer/HCT-116 cell line
Wainunuamide	Cyclic hexapeptide	*Stylotella aurantium*	Cancer/A2780 ovarian, K562 leukemia cancer cells
Leucamide A	Cyclic hexapeptide	*Leucetta microraphis*	Cancer/Tumor cell lines HM02, HepG2, Huh7
Axinellin C	Cyclic octapeptide	*Stylotella aurantium*	Cancer/A2780 ovarian, K562 leukemia cancer cells
Milnamide D	Linearpeptide	*Cymbastela* sp.	Cancer/HCT-116 cells
Kapakahines E–G	—	*Cribrochalina olemda*	Cancer/P388 murine leukemia cells
Didmolamides A-B	Cyclic hexapeptides	*Didemnum molle*	Cancer Tumor cell lines (A549, HT29 and MEL28)
Bistratamides E–J	Cyclic hexapeptides	*Lissoclinum bistratum*	Cancer/Human colon tumor (HCT-116) cell line
Milnamide C	—	*Auletta* sp.	Cancer/MDA-MB-435cancer cells
Scleritodermin A	Cyclic peptide	*Scleritoderma nodosum*	Cancer

NRPs agents	Chemical class	Origin	Target
Microcionamids A-B	—	*Clathria abietina*	Cancer/Human breast tumor cell lines MCF-7 and SKBR-3
Kendarimide A	Linear peptide	*Haliclona* sp.	Cancer/KB-C2 cells
Phakellistatin 14	Cyclo heptapeptide	*Phakellia* sp.	Cancer/Murine lymphocytic leukemia P388 cell line
Polytheonamides A-B	Polypeptides	*Theonella swinhoei*	Cancer/P388 murine leukemia cells
Neopetrosiamides A-B	Tricyclic peptides	*Neopetrosia* sp.	Cancer
Seragamides A–F	Depsipeptides	*Suberites japonicus*	Cancer
Theopapuamide	Cyclic depsipeptide	*Theonella swinhoei*	Cancer/CEM-TART, HCT-116 cell lines
Azumamide A-E	Cyclo tetrapeptides	*Mycale izuensis*	Cancer
Callyaerin G	Cyclic peptide	*Callyspongia aerizusa*	Cancer/Mouselymphoma cell line (L5178Y) and HeLa cells
Stylopeptide 2	Cyclo decapeptide	*Stylotella* sp.	Cancer/BT-549 and HS578T breast cancer cell lines
Ciliatamides A-C	Lipopeptides	*Aaptos ciliate*	Cancer/HeLa cells
Diazonamides C–E	Macrocyclic peptides	*Diazona* sp.	Cancer/Human tumor cell lines (A549, HT29, MDA-MB231)
Rolloamide A-B	Cyclic heptapeptides	*Eurypon laughlini*	Cancer
Euryjanicin A	Cycloheptapeptide	*Prosuberites laughlini*	Cancer
Callyaerin A–F and H	Cyclic peptides	*Callyspongia aerizusa*	Cancer/L5178Y cell line
Papuamides E-F	Depsipeptides	*Melophlus* sp.	Cancer/Brine shrimp
Stylissamide X	Octapeptide	*Stylissa* sp.	Cancer/HeLa cells
Gombamide A	Hexapeptide	*Clathria gombawuiensis*	Cancer/K562 and A549 cell lines
Microspinosamide	Cyclic depsipeptide	*Sidonops microspinosa*	HIV
Neamphamide A	Cyclic depsipeptide	*Neamphius huxleyi*	HIV
Mirabamides A-D	Cyclic depsipeptide	*Siliquariaspongia mirabilis*	HIV
Homophymine A	Cyclodepsipeptide	*Homophymia* sp.	HIV/PBMC cell line
Celebeside A-C	Depsipeptides	*Siliquariaspongia mirabilis*	HIV/Colon carcinoma (HCT-116) cells
Theopapuamides B–D, Mutremdamide A, Koshikamides C-H	Cyclic depsipeptide	*Theonella* sp.	HIV
Ceratospongamide	Cyclic heptapeptide	*Sigmadocia symbiotica*	Inflammation
Halipeptin A-B	Cyclic depsipeptide	*Haliclona sp.*	Inflammation

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NRPs agents	Chemical class	Origin	Target
Perthamide C-D	Cyclopeptide	*Theonella swinhoei*	Inflammation
Solomonamide A- B	Cyclic peptide	*Theonella swinhoei*	Inflammation
Stylissatin A	Cyclic peptide	*Stylissa massa*	Murine macrophage RAW264.7
Dicynthaurin	—	*Halocynthia aurantium*	Antimicrobial
Nagahamide A	Depsipeptide	*Theonella swinhoei*	Antibacterial
Plicatamide	Octapeptide	*Styela plicata*	Antimicrobial
Callipeltins	—	*Latrunculia sp.*	*Antifungal/Candida albicans*
Citronamides A- B	—	*Citronia astra*	*Antifungal/Saccharomyces cerevisiae*
Renieramide	Cyclic tripeptide	*Reniera* sp.	—
Phoriospongins A-B	Depsipeptide	*Phoriospongia* sp. and *Callyspongia bilamellata*	Nematocidal/*Haemonchus contortus*

Table 4.
Agents produced from marine sponges and tunicates which are based on NRPs [7].

In the NCBI database, there are currently about 1.164 distinct non-ribosomal peptides that form over 500 different monomers including both proteinogenic and non-proteinogenic L- and D-amino acids, as well as amines and carboxylic acids. These complex secondary metabolites' linear, cyclic, branching, or other complicated primary structures are frequently altered to enhance clinical qualities and/or bypass resistance mechanisms. Indeed, the nucleotide sequence modification of a native NRPS gene or mixing modules from multiple NRPSs makes them more efficient with pharmacological properties. Several bioengineering and molecular techniques have been developed during the last few decades to produce modified NRPs with improved physicochemical characteristics and bioactivity [13].

5. Conclusion

In this chapter, we discussed the significance, synthesis, and application areas of NRPs-based agents, which have received a lot of interest as a new source of pharmaceutical agents. NRPs with unique chemical structures and diverse biological actions, such as antibacterials (penicillin, vancomycin), anticancer compounds (bleomycin), and immunosuppressants (cyclosporine), have been researched as novel compounds for new drug discovery and development throughout the last several decades. *In vitro* bioassays and the transfer of biosynthetic gene clusters of NRPs have been the focus of the majority of these studies. For the development of NRPs drugs with improved pharmacological properties, genetic manipulation and molecular approaches will allow the rapid construction of new NRPSs containing specific point mutations or exchanged domains.

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