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Review Article

Temporins: An Approach of Potential Pharmaceutic Candidates

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Abstract

Antimicrobial peptides (AMPs), also known as host defense peptides, are small and mostly polycationic molecules that form part of the innate immune response. There are currently more than 3000 experimentally reported AMPs. Particularly in frogs, the temporin family has been discovered as potential AMPs. The aim of this work is to review the latest publications about this class of peptides, discuss their properties, and present an update of the last studies and new discoveries in the field. More than 130 temporins have been identified in this family. The most studied temporins are temporin A (TA), temporin B (TB), and temporin L (TL). These peptides showed antimicrobial activity against gram-negative, gram-positive bacteria and fungi. Since the discovery of temporins in 1996, several groups of researchers isolated different peptides from various species of frogs that were included as members of this family. Although antimicrobial activity of many temporins has not been analyzed yet, most of them showed antimicrobial and antifungal activities. A combination of nanotechnology and AMPs for temporins in different antimicrobial treatments could be a promising alternative for resistant pathogens. These studies demonstrate that, even with the advancement in scientific research on the composition and antimicrobial activity of temporins, further studies are necessary to wholly understand their components and mechanisms of action.

Keywords: antifungals; antimicrobial peptides; drug delivery; frog

Antimicrobial peptides (AMPs), also known as host defense peptides (HDPs), are small and mostly polycationic molecules that form part of the innate immune response. Most of the AMPs share common features, such as small size, with cationic and hydrophobic sequences within a linear or cyclic structure. The AMPs can inhibit or eliminate bacteria at micromolar concentrations, often by non-specific mechanisms. Moreover, AMPs can kill antibiotic-resistant bacteria [1].

Currently, there are more than 3000 reported AMPs, including both natural peptides produced by living organisms from protozoa to animals and plants and artificial synthetic peptides. The vast number of compounds with known biologic activities are listed in specialized AMP databases such as the Antimicrobial Peptide Database 3 (APD3, [2]), the Collection of Anti-Microbial Peptides (CAMPR3, [3]) and the Database Linking Antimicrobial Peptides (LAMP, [4]). This list contains 44 AMP families, including well- known AMPs such as defensins, brevinines, esculentins, japonicins, nigrocins, palustrins, ranacyclins, ranatuerins, cecropins, temporins, magainins, and dermaseptins, some of them studied recently [5].

Frogs (family Ranidae) synthesize a remarkably diverse range of antimicrobial peptides that are released from the granular glands to skin secretions in a very high concentration and a holocrine manner as a consequence of stress or tissue injury. These peptides serve to protect the frog against invasion by a variety of pathogenic micro-organisms and represent a component of the innate immunity of these organisms [6,7].

Within this arsenal of defensive peptides, some members of the temporin family were described [8]. These hydrophobic, C-terminally α-amidated peptides were first identified in the skin of the Asian frog *Rana erythraea* and the European frog *R. esculenta* (reclassified as a hybrid between *Pelophylax*

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ridibundus and *P. lessonae*) on the basis of their hemolytic activity [9]. In 1996, Simmaco et al. [10] identified these peptides in the skin of the European red frog *R. temporaria*. Later, they were also discovered in frogs of Northern America and Eurasian origin as well as in the venom of wasps [11,12].

Previous reports reviewed only 75 temporins. In addition, in the last five years, new members of this family were described in different frog species and studied for antimicrobial activity. Moreover, new approaches for pharmacologic discoveries, drug delivery, and nanotechnology have been applied using temporins because of their interesting properties and the well-known antimicrobial activity. In particular, two of the most studied temporins (temporin A and B) were used recently as potential candidates in medical and molecular new researches.

The aim of this work is to review the latest publications concerning this family of peptides, discuss their properties and potential applications, and provide an update of the last studies and new discoveries in the field.

Temporins

Initially, temporins were described as ''*Vespa*-like'' because of their structural similarity to the short (13-14 amino acids) peptides with chemotactic and histamine-releasing properties isolated from the venom of wasps of the genus *Vespa* [12]. Many peptides from the wasp venom have been used as antimicrobial drugs for many purposes—for example, intravascular stents infections [13], and other bacterial [14,15] or fungal [16]. Temporins present some similarities to these *Vespa* peptides—for example, low molecular masses, hydrophobic properties, and antimicrobial activity. The derived amino acid sequence for temporins is FLP(I/L)IASLL(S/G) KLL-NH_2, and the general amino acid type sequence is $X_1X_2X_3X_4X_5X_6Y_7X_8X_9Y_{10}Y_{11}{}^{+}X_{12}X_{13}$-$NH_2$ (X = hydrophobic, Y = hydrophilic and Y^+ = charged amino acid).

Hydrophobic residues represent 70% of the peptide sequence, leucine being the most abundant amino acid. In addition, X_2, X_3, X_9, and X_{13} are highly conserved amino acids [17]. Most of the temporins adopt an α-helical conformation in hydrophobic environments [18].

More than 130 members of the temporin family have been identified in the skins of Eurasian, Asian, and North American ranids (Table 1, [19–66]). The temporins are among the most highly variable of all antimicrobial peptides. Most contain a single basic residue (generally Lys) giving a charge of +1, but several temporins lack this structural feature. Temporin L, 1-CEa, 1-CEc, 1-CSd, CPa, 1Dra, 1DRb, HN1, 1Lb, 1Lc, LTb, LT2, 1Ola, PRc, PTa, Ra, RN3, and 1TGb contain two basic residues. In addition, 24 temporins have no charge and three (CDYa, CDYc, and 1Ja) have acidic amino acids, with negative charge (Fig. 1). They are C-terminally α-amidated, and their amino acid sequences are composed mainly by hydrophobic residues.

Temporins present interesting properties that make them good candidates for biologic investigation. Some of these properties are: (1) They are small amphipathic α-helical AMPs (typically 13 amino acids); (2) they have low positive net charge at a neutral pH (between 0 and +3); (3) their short sequence provides low cost-efficient chemical synthesis; (4) some of the temporins act efficiently against a wide range of pathogens (bacteria, viruses, filamentous fungi, yeasts, and protozoa) and are not toxic to mammalian cells; (5) their mechanism of action is mainly based on cytoplasmic membrane perturbation, but in a different way from that proposed for the majority of cationic α-helical AMPs; (6) some temporins display antimicrobial and chemotactic activities, immunomodulatory effects, and preserve biologic function in serum; (7) they display synergistic action when combined with conventional antibiotic agents; (8) they show in vivo efficacy as topical prophylactic agents against infections and in vivo anti-endotoxic effects [67,68].

Although most temporins are inhibitors of gram-positive bacteria, some peptides from this family seem to be atypical in displaying activity against reference strains of several clinically relevant gram-negative species and against the opportunistic yeast pathogen *Candida albicans* (Fig 2).

The mode of action of the temporin is still uncertain, but some researchers found that the spatial conformation is essential to penetrate the cell membrane. In general, temporins adopt an α-helix conformation, although this can change in different mediums [67,69–71].

Temporins A, B, and L

Temporins A, B, and L are the most studied as antimicrobial peptides.

Temporin A

Structure–activity studies with temporin A (TA, FLPLIGRVLSGIL-NH_2) indicated that a hydrophobic N-terminal residue and bulky hydrophobic residues at positions 5 and 12 are important determinants of antibacterial activity [68]. The TA is preferentially active against gram-positive bacteria and has a moderate hemolytic activity [72]. The TA, together with temporin L, is one of the most studied peptides, and it was found to show interesting antimicrobial activity.

For example, together with temporin 1P from *R. pipiens* and a wasp temporin, TA successfully inhibited the growth of the fungus *Batrachochytrium dendrobatidis*, a pathogen associated with global amphibian declines. In this case, the results obtained with the peptides indicated that the ability to penetrate membranes and form an α-helical structure was important for their effectiveness against the pathogen [73]. Also, this peptide showed antimicrobial activity against *Staphylococcus aureus*, *Enterococcus faecium,* and *E. faecalis* [74,75].

Wade et al. [76] synthetized a hybrid peptide (CA(1-7)TA(2-9)NH_2 (CATA)), containing portions of the amino-acid sequences of TA and cecropin A (CA) and evaluated its hemolytic, anticoagulant, and antifungal properties. Cecropin A is a gene encoded antibiotic peptide that was originally isolated from the silkmoth *Hyalophora cecropia* larval hemolymph. In previous works, portions of amino acids of CA were combined with melittin, a peptide whose sequence of the amino terminal portion is similar to TA. They found that both TA and CATA are not hemolytic at concentrations at which TA yields antimicrobial effects (less than 12 μM). The CATA has a weaker but still significant activity against the chytrid fungus *Batrachochytrium dendrobatidis*. While TA does not affect coagulation times at the concentrations studied, CATA inhibits coagulation at all concentrations tested [76].

In another study, TA structure was modified to make three different analogues: A monomeric carboxy peptide (TAc), a dimeric temporin (TAd), and a synthesized analogue using a

Table 1. Updated List of Temporins Described since First Discovery in 1996

Temporin	*Amino acid sequence*	*No. amino acids*	*Frog specie*	*Charge*	*Basic amino acids*	*Acidic amino acids*	*Reference*
A	FLPLIGRVLSGIL-NH_2	13	*Rana temporaria*	+1	1	0	[10]
B	LLPIVGNLLKSLL-NH_2	13	*Rana temporaria*	+1	1	0	[10]
C	LLPILGNLLNGLL-NH_2	13	*Rana temporaria*	0	0	0	[10]
D	LLPIVGNLLNSLL-NH_2	13	*Rana temporaria*	0	0	0	[10]
E	VLPIIGNLLNSLL-NH_2	13	*Rana temporaria*	0	0	0	[10]
F	FLPLIGKVLSGIL-NH_2	13	*Rana temporaria*	+1	1	0	[10]
G	FFPVIGRILNGIL-NH_2	13	*Rana temporaria*	+1	1	0	[10]
H	LSPNLLKSLL-NH_2	10	*Rana temporaria*	+1	1	0	[10]
K	LLPNLLKSLL-NH_2	10	*Rana temporaria*	+1	1	0	[10]
L	FVQWFSKFLGRIL-NH_2	13	*Rana temporaria*	+2	2	0	[10]
1ARa	FLPIVGRLISGLL-NH_2	13	*Rana areolata*	+1	1	0	[19]
1AUa	PLPIIGQLLSGLL-NH_2	13	*Rana aurora aurora*	0	0	0	[20]
ALa	FLPIVGKLLSGLSGLL-NH_2	16	*Amolops loloensis*	+1	1	0	[21]
Alb	FLPIVGKLLSGLL-NH_2	13	*Amolops loloensis*	+1	1	0	[22]
ALc	LLPIVGKGLSGKL-NH_2	13	*Amolops loloensis*	+2	2	0	[22]
ALd	FLPIAGKLLSGLSGLL-NH_2	16	*Amolops loloensis*	+1	1	0	[22]
ALe	FFPIVGKLLFGLSGLL-NH_2	16	*Amolops loloensis*	+1	1	0	[22]
ALf	FFPIVGKLLSGLSGLL-NH_2	16	*Amolops loloensis*	+1	1	0	[22]
ALg	FFPIVGKLLFGLFGLL	16	*Amolops loloensis*	+1	1	0	[22]
ALh	FLPIVGKLLSGLSGLS-NH_2	16	*Amolops loloensis*	+1	1	0	[22]
ALi	FFPIVGKLLSGLL-NH_2	13	*Amolops loloensis*	+1	1	0	[22]
ALj	FFPIVGKLLFGLL-NH_2	13	*Amolops loloensis*	+1	1	0	[22]
ALk	FFPIVGKLLS-NH_2	10	*Amolops loloensis*	+1	1	0	[22]
1BYa	FLPIIAKVLSGLL-NH_2	13	*Rana boylii*	+1	1	0	[23]
1Ca	FLPFLAKILTGVL-NH_2	13	*Rana clamitans*	+1	1	0	[24]
1Cb	FLPLFASLIGKLL-NH_2	13	*Rana clamitans*	+1	1	0	[24]
1Cc	FLPFLASLLTKVL-NH_2	13	*Rana clamitans*	+1	1	0	[24]
1Cd	FLPFLASLLSKVL-NH_2	13	*Rana clamitans*	+1	1	0	[24]
1Ce	FLPFLATLLSKVL-NH_2	13	*Rana clamitans*	+1	1	0	[24]
1CEa	FVDLKKIANIINSIF-NH_2	15	*Rana chensinensis*	+1	1	0	[25]
1CEb	ILPILSLIGGLLGK-NH_2	14	*Rana chensinensis*	+1	1	0	[25]
1CEc	IIPLPLGYFAKKT-NH_2	13	*Rana chensinensis*	+2	2	0	[25]
CDYa	VLPLVGNLLNDLL-NH_2	13	*Rana dybowskii*	-1	0	1	[26]
CDYb	ILPILAPLIGGLL-NH_2	13	*Rana dybowskii*	0	0	0	[26]
CDYc	VLPLVGNLLNDLL-NH_2	13	*Rana dybowskii*	-1	0	1	[26]
CDYd	FIGPLISALASLFKG-NH_2	15	*Rana dybowskii*	+1	1	0	[27a]
CDYe	FIGPIISALASLFGG-NH_2	15	*Rana dybowskii*	0	0	0	[27b]
CG1	FLPFVGNLLKGLL-NH_2	13	*Amolops chunganensis*	+1	1	0	[28]
CG2	FFPIVGKLLSGLF-NH_2	13	*Amolops chunganensis*	+1	1	0	[28]
CG3	FLPIVGKLLSGLF-NH_2	13	*Amolops chunganensis*	+1	1	0	[28]
CG4	FLPILGNLLNGLL-NH_2	13	*Amolops chunganensis*	0	0	0	[28]
CG5	FLPFVGNLLNGLL-NH_2	13	*Amolops chunganensis*	0	0	0	[28]
1CSa	FLPIVGKLLSGLL-NH_2	13	*Rana cascadae*	+1	1	0	[29]
1CSb	FLPIIGKLLSGLL-NH_2	13	*Rana cascadae*	+1	1	0	[29]
1CSc	FLPLVTGLLSGLL-NH_2	13	*Rana cascadae*	0	0	0	[29]
1CSd	NFLGTLVNLAKKIL-NH_2	14	*Rana cascadae*	+2	2	0	[29]
CPa	IPPFIKKVLTTVF-NH_2	13	*Lithobates capito*	+2	2	0	[30]
CPb	FLPIVGRLISGIL-NH_2	13	*Lithobates capito*	0	0	0	[30]
1DRa	HFLGTLVNLAKKIL-NH_2	14	*Rana aurora draytonii*	+2	2	0	[31]
1DRb	NFLGTLVNLAKKIL-NH_2	14	*Rana aurora draytonii*	+2	2	0	[31]
1DRc	FLPIIASVLSSLL-NH_2	13	*Rana aurora draytonii*	0	0	0	[31]
1DYa	FIGPIISALASLFG- NH_2	14	*Rana dybowskii*	0	0	0	[32]
1Ec	FLPIIAGLLSKLF-NH_2	13	*Rana esculenta*	+1	1	0	[33]
1Ee	FLPVIAGVLSKLF-NH_2	13	*Pelophylax kl. esculentus*	+1	1	0	[34]
1Ga	SILPTIVSFLSKVF-NH_2	14	*Rana grylio*	+1	1	0	[35]
1Gb	SILPTIVSFLSKFL-NH_2	14	*Rana grylio*	+1	1	0	[35]
1Gc	SILPTIVSFLTKFL-NH_2	14	*Rana grylio*	+1	1	0	[35]
1Gd	FILPLIASFLSKFL-NH_2	14	*Rana grylio*	+1	1	0	[35]
GH	FLPLLFGAISHLL-NH_2	13	*Hylarana guentheri*	0	0	0	[36]
Gua	FLQHIIGALSHIF-NH_2	13	*Hylarana guntheri*	0	0	0	[37]
Gub	FLPLLFGAISHIL-NH_2	13	*Hylarana guntheri*	+1	1	0	[37]
Guc	FFPLIFGALSSILPKIL-NH_2	17	*Hylarana guntheri*	+1	1	0	[37]
1HK	SIFPAIVSFLSKFL-NH_2	14	*Rana heckscheri*	+1	1	0	[38]

(*continued*)

Table 1. (Continued)

Temporin	*Amino acid sequence*	*No. amino acids*	*Frog specie*	*Charge*	*Basic amino acids*	*Acidic amino acids*	*Reference*
HN1	AILTTLANWARKFL-NH_2	14	*Odorrana hainanensis*	+2	2	0	[39]
HN2	NILNTIINLAKKIL-NH_2	14	*Odorrana hainanensis*	+2	2	0	[39]
1Ja	ILPLVGNLLNDLL-NH_2	13	*Rana japonica*	-1	0	1	[40]
1La	VLPLISMALGKLL-NH_2	13	*Rana luteiventris*	+1	1	0	[41]
1Lb	NFLGTLINLAKKIM-NH_2	14	*Rana luteiventris*	+2	2	0	[41]
1Lc	FLPILINLIHKGLL-NH_2	14	*Rana luteiventris*	+2	2	0	[41]
LK1	FFPLLFGALSSMMPKLF-NH_2	17	*Limnonectes kuhlii*	+1	1	0	[42]
LTa	FFPLVLGALGSILPKIF-NH_2	17	*Hylarana latouchii*	+1	1	0	[43]
LTb	FIITGLVRGLTKLF-NH_2	14	*Hylarana latouchii*	+2	2	0	[43]
LTc	SLSRFLSFLKIVYPPAF-NH_2	17	*Hylarana latouchii*	+2	2	0	[43]
LTe	FLAGLIGGLAKML-NH_2	13	*Hylarana latouchii*	+1	1	0	[44]
LT1	FLPGLIAGIAKML-NH_2	13	*Hylarana latouchii*	+1	1	0	[45]
LT2	FLPIALKALGSIFPKIL-NH_2	17	*Hylarana latouchii*	+2	2	0	[45]
1M	FLPIVGKLLSGLL-NH_2	13	*Rana muscosa*	+1	1	0	[46]
1Oa	FLPLLASLFSRLL-NH_2	13	*Rana ornativentris*	+1	1	0	[47]
1Ob	FLPLIGKILGTIL-NH_2	13	*Rana ornativentris*	+1	1	0	[47]
1Oc	FLPLLASLFSRLF-NH_2	13	*Rana ornativentris*	+1	1	0	[47]
1Od	FLPLLASLFSGLF-NH_2	13	*Rana ornativentris*	0	0	0	[47]
1Oe	ILPLLGNLLNGLL-NH_2	13	*Rana ornativentris*	0	0	0	[48]
1Of	SLLLKGLASIAKLF-NH_2	14	*Rana ornativentris*	+1	1	0	[48]
1Og	FLSSLLSKVVSLFT-NH_2	14	*Rana ornativentris*	+1	1	0	[48]
1OLa	FLPFLKSILGKIL-NH_2	13	*Rana okaloosae*	+2	2	0	[49]
1OLb	FLPFFASLLGKLL-NH_2	13	*Rana okaloosae*	+1	1	0	[49]
1P	FLPIVGKLLSGLL-NH_2	13	*Rana pipiens*	+1	1	0	[41]
1PLa	FLPLVGKILSGLI-NH_2	13	*Rana palustris*	+1	1	0	[50]
1PRa	ILPILGNLLNGLL-NH_2	13	*Rana pirica*	0	0	0	[51]
1PRb	ILPILGNLLNSLL-NH_2	13	*Rana pirica*	0	0	0	[51]
PRa	FLPILGNLLSGLL-NH_2	13	*Rana pretiosa*	0	0	0	[52]
PRb	FLPIITNLLGKLL-NH_2	13	*Rana pretiosa*	+1	1	0	[52]
PRc	NFLDTLINLAKKFI-NH_2	14	*Rana pretiosa*	+1	1	1	[52]
PTa	FFGSVLKLIPKIL-NH_2	13	*Hylarana picturata*	+2	2	0	[53]
Ra	FLKPLFNAALKLLP-NH_2	14	*Rana ridibunda*	+2	2	0	[54]
Rb	FLPVLAGVLSRA-NH_2	12	*Rana ridibunda*	+1	1	0	[54]
1Re	FLPGLLAGLL-NH_2	10	*Pelophylax kl. esculentus*	0	0	0	[34]
RN1	FLPLVLGALSGILPKIL-NH_2	17	*Rana nigrovittata*	+1	1	0	[55]
RN2	FFPLLFGALSSLLPKLF-NH_2	17	*Rana nigrovittata*	+1	1	0	[55]
RN3	FFPLLFGALSSHLPKLF-NH_2	17	*Rana nigrovittata*	+2	2	0	[55]
1Sa	FLSGIVGMLGKLF-NH_2	13	*Pelophylax saharica*	+1	1	0	[56]
1Sb	FLPIVTNLLSGLI-NH_2	13	*Pelophylax saharica*	0	0	0	[56]
1Sc	FLSHIAGFLSNLF-NH_2	13	*Pelophylax saharica*	+1	1	0	[56]
1SKa	FLPVILPVIGKLLNGIL-NH_2	17	*Rana sakuraii*	+1	1	0	[57]
1SKb	FLPVILPVIGKLLSGIL-NH_2	17	*Rana sakuraii*	+1	1	0	[57]
1SPa	FLSAITSILGKFF-NH_2	13	*Rana septentrionalis*	+1	1	0	[58]
1SPb	FLSAITSLLGKLL-NH_2	13	*Rana septentrionalis*	+1	1	0	[58]
1SPc	FLSAITSILGKLF-NH_2	13	*Rana septentrionalis*	+1	1	0	[58]
SHa	FLSGIVGMLGKLF-NH_2	13	*Pelophylax saharica.*	+1	1	0	[59]
SHb	FLPIVTNLSGLL-NH_2	12	*Pelophylax saharica.*	0	0	0	[59]
SHc	FLSHIAGFLSNLF-NH_2	13	*Pelophylax saharica*	+1	1	0	[59]
SHd	FLPAALAGIGGILGKLF-NH_2	17	*Pelophylax saharica*	+1	1	0	[60]
SHf	FFFLSRIF-NH_2	8	*Pelophylax saharica*	+1	1	0	[23]
SN1	FFPFLLGALGSLLPKIF-NH_2	17	*Hylarana spinulosa*	+1	1	0	[61]
SN2	FITGLIGGLMKAL-NH_2	13	*Hylarana spinulosa*	+1	1	0	[61]
SN3	FISGLIGGLMKAL-NH_2	13	*Hylarana spinulosa*	+1	1	0	[61]
SN4	FITGLISGLMKAL-NH_2	13	*Hylarana spinulosa*	+1	1	0	[61]
SN5	FFPLVLGALGSILPKIF-NH_2	17	*Hylarana spinulosa*	+1	1	0	[61]
1TGa	FLPILGKLLSGIL-NH_2	13	*Rana tagoi*	+1	1	0	[62]
1TGb	AVDLAKIANKVLSSLF-NH_2	16	*Rana tagoi*	+1	2	1	[63]
1TGc	FLPVILPVIGKLLSGIL-NH_2	17	*Rana tagoi*	+1	1	0	[63]
1TSa	FLGALAKIISGIF-NH_2	13	*Rana tsushimensis*	+1	1	0	[64]
1TSb	FLPLLGNLLNGLL-NH_2	13	*Rana tsushimensis*	0	0	0	[64]
1TSc	FLPLLGNLLRGLL-NH_2	13	*Rana tsushimensis*	+1	1	0	[64]
1TSd	FLPLLASLIGGML-NH_2	13	*Rana tsushimensis*	0	0	0	[64]
1VE	FLPLVGKILSGLI-NH_2	13	*Rana (Odorrana) versabilis*	+1	1	0	[65]

(*continued*)

Table 1. (Continued)

Temporin	*Amino acid sequence*	*No. amino acids*	*Frog specie*	*Charge*	*Basic amino acids*	*Acidic amino acids*	*Reference*
1Va	FLSSIGKILGNLL-NH_2	13	*Rana virgatipes*	+1	1	0	[66]
1Vb	FLSIIAKVLGSLF-NH_2	13	*Rana virgatipes*	+1	1	0	[66]
1Vc	FLPLVTMLLGKLF-NH_2	13	*Rana virgatipes*	+1	1	0	[66]
Wa	FISKIASLGAGVLX-NH_2	14	*Lithobates warszewitschii*	+1	1	0	[30]

The table shows the sequence, number of amino acids, positive, negative, and net charge of each temporin. Those amino acids in red (K, H, and R) correspond to basic amino acids, and those in green (D, E) correspond to acidic amino acids.

new branching unit 3-*N,N*-di(3-aminopropyl)amino propanoic acid (DAPPA), which allows building of the parallelly symmetric α-helical structures. These analogues were tested against *S. aureus* (gram-positive) and *Escherichia coli* (gram-negative). Both TA and TAd completely inhibited the growth of *S. aureus*, whereas TAc did not show any inhibitory activity. The TAd displayed antibacterial effect against *E. coli*, whereas monomeric TA did not show any activity at concentration higher than 20 mM. The results indicated that these structural modifications improved the antibacterial properties of TA especially because of the increase of the net charge of the peptide from +2 to +4 [77].

The TA enantiomer (with D-amino acids, D-TA) also showed good antimicrobial activity against bacteria, and the replacement of some amino acids improved the activity [68]. In another study, the D-TA and the reverse TA sequence (Rev-TA) were compared with TA. The TA induced monocyte migration, while both TA analogues did not, suggesting that TA-induced monocyte migration is based on a chiral interaction [78].

Other studies performed with temporin A, B, D, and H have shown that in Lysogeny broth (LB) medium, temporins D and H were completely inactive, whereas TA and TB showed a higher activity against the gram-positive bacterial strain than against the gram-negative one. In addition, TA and TB also caused total reduction in colony-forming units of *S. aureus* [67].

In addition, Magnoni et al. [79] found an anti-*Leishmania* activity at micromolar concentrations for TA and TB with no cytolytic activity against human erythrocytes. The ability of temporins to kill *Leishmania* amastigotes was assayed by inhibition of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction test [79]. Both temporins preserved their activity against the mammalian intracellular form of the parasite.

They also investigated whether the mode of action of temporins against *Leishmania* amastigotes was similar to that found for promastigotes. They found that TA and TB could induce the permeation of the amastigote membrane, although TB was more active than TA. In addition, both temporins induced the permeation of the membrane of amastigotes similarly to the promastigotes [67]. Finally, experiments with TA found that this peptide not only is suitable as an antimicrobial peptide but also can be administrated topically in mice wounds together with antibiotic agents, generating a significant bacterial growth inhibition and acceleration of wound repair process [80].

To improve AMPs administration, new polymeric carriers can be used as highly controlled release devices. Piotrowska et al. [81] chose the peptides citropin 1.1 (CIT) and TA for their experiments. They found that the release rate of the active pharmaceutic ingredients (APIs) was strongly dependent on the API characteristics and the matrix microstructure. They investigated the effect of the carrier polymer microstructure in both peptide kinetics release in vitro. Results suggested these polymeric devices as good therapeutic carrier candidates and TA as a prospective therapeutic option [81].

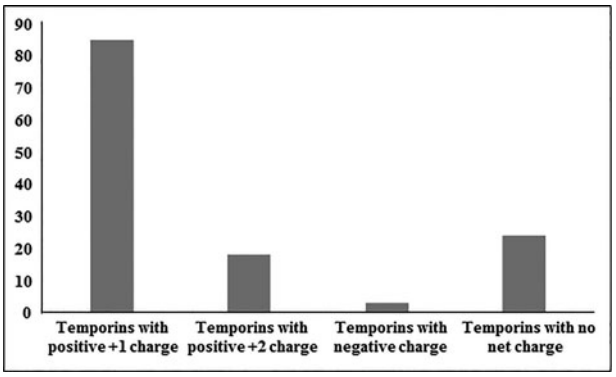

FIG. 1. Number of temporins with +1, +2, -1, and 0 charge. Most of the temporins (85 peptides) present +1 charge, 24 temporins have no net charge, 18 have +2, and only three temporins have negative charge.

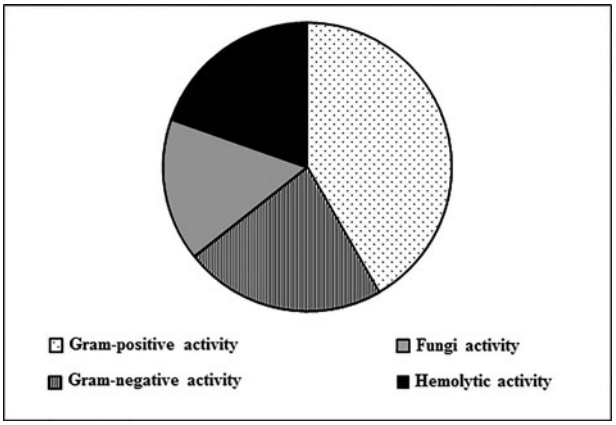

FIG. 2. Antimicrobial and hemolytic activity of the temporins. From 85 temporins analyzed against gram-positive bacteria, 83 showed positive activity; from 79 temporins analyzed against gram-negative bacteria, 45 showed positive activity; and 32 of 65 temporins showed good antimicrobial activity against fungi. From 57 temporins, 39 presented hemolytic activity.

The TA applications were not limited to antimicrobial activities. Musale et al. [82] found that TA, together with temporin F (TF) and G (TG), produced concentration-dependent stimulation of insulin release from BRIN-BD11 rat clonal β-cells at concentrations ≥1 nM, without cytotoxicity at concentrations up to 3 mcM. In these experiments, TA was the most effective. Also, TA together with TF protected BRIN-BD11 cells against cytokine-induced apoptosis [82].

Temporin B

Malgieri et al. [83] elucidated mechanisms of action of temporin B (TB, LLPIVGNLLKSLL-NH_2) synthetic derivatives. Some of these peptide derivatives showed gram-negative and gram-positive inhibition with no hemolytic activity. Marcocci et al. [84] recently demonstrated for the first time the in vitro anti-herpes simplex virus type 1 (HSV-1) activity of TB. A significant antiviral activity was observed in a pre-incubation of HSV-1 with 20 mcg/mL of TB. When TB was added to HSV-1-infected cells, viral titer was significantly reduced. Moreover, the highest inhibition was obtained by pre-incubation of HSV-1 with the peptide for 1 h at 37°C, demonstrating its viricidal activity [84].

In addiion, a D form of TB has been used together with TB to test the antimicrobial activity compared with CAMEL peptide (CA(1–7)M(2–9)NH_2). This last peptide is a 15-residue hybrid amide peptide with seven amino acids that are derived from the sequence of cecropin A (silkmoth *Hyalophora cecropia* larvae) and eight amino acids from the sequence of melittin (honey-bee venom). The results showed that the antimicrobial activity of the D form of temporin B was detected after 30 min, and no activity was detected after 24, 48, and 72 h. The D isomer of temporin B was inactivated more slowly in feces than the L isomer [85].

Another study evaluated the role of each amino acid on the peptide activity of TB. Avitable et al. [86] performed a complete Ala scanning of TB by substituting one or two amino acids with alanine residues to obtain a series of TB analogues. Best results were obtained with the analogue TB_KKG6A (KKLLPIVANLLKSLLNH_2) that showed antimicrobial activity against gram-positive and gram-negative bacteria at a concentration of 5 mcM [86]. This peptide, together with other analogues, was used to find antimicrobial activity against *Pseudomonas aeruginosa*. One of the TB analogues, TBKKG6A, exhibited strong antimicrobial activity [87].

In addition, enhancing the hydrophobicity of the N-terminus and the cationicity of the C-terminus in TB improves its membrane activity and potency against both gram-negative and gram-positive bacteria. In contrast, enhancing the cationicity of the N-terminus abrogates its ability to trigger channel conductance and renders it ineffective against *S. aureus* while nevertheless enhancing its potency against *E. coli* [70].

In other studies concerning infection of human keratinocytes with *S. aureus*, TB and TA were used to improve the treatment against this bacterium. Results showed that TB killed ATCC-derived (ATCC: American Type Culture Collection) and multi-drug–resistant clinical isolates *of S. aureus* within infected human keratinocytes at a non-toxic concentration of 16 mcM within 2 h and without injuring the host cells, whereas a weaker effect was displayed by TA. With the combination of antibacterial and wound-healing activities, TA and TB seemed to act as multi-functional mediators of innate immunity in humans [88]. In addition, TB acted synergistically with other reagents, such as cysteine and ethylenediaminetetraacetic acid. These combinations improved the antimicrobial activity of the temporin against *S. epidermidis* biofilms [89].

Lipopolysaccharides (LPS) have been important in temporin studies because of the interaction between them and the fact that LPS are the major structural component of the outer membrane of gram-negative bacteria. The LPS usually are used as models for molecular dynamics to study the antimicrobial activity of the temporins—for example, TB [90]. Also, LPS have been used in different studies using temporin L (see below).

These results make TB a promising alternative as an antibacterial drug, although its toxicity still needs more research, especially in mammalian cells. It is well known that AMPs represent promising therapeutic agents against bacterial and fungal pathogens, although one of their principal limitations is their cytotoxicity to eukaryotic cells and erythrocytes [91,92]. The TB is not an exception. Some alternatives to solve this problem have been proposed to improve its pharmaceutic development, such as TB encapsulation into chitosan nanoparticles [93].

Temporin L

Temporin L (TL, FVQWFSKFLGRIL-NH_2) has a large antimicrobial activity, especially for fungal pathogens and gram-negative bacteria, while temporins are generally gram-positive inhibitors. This temporin has potential anti-endotoxin properties and can strongly bind to purified *E. coli* LPS and interfere with its biologic activities in two rat models of gram-negative septic shock, protecting the animals against lethal endotoxemia [72].

The mechanism of action of TL (together with TB) to penetrate the phospholipid bilayer has been studied in different ways, and it seems the peptides penetrate the membrane through the formation of fibrillar protrusions [94]. The interaction of TL (and TB) with bacteria membrane has also been studied using spectroscopic techniques [95]. This peptide displays the highest antimicrobial potency among temporins tested to date and has strong affinity for lipid membranes and for the LPS component of the gram-negative outer membrane [72].

As stated earlier, LPS are important in temporin studies. The temporins not only interact with LPS but also can exhibit a synergistic effect when more than two temporins are combined [96,97]. As found for TA, TL also can be administrated intraperitoneally with piperacillin and imipenem, two β-lactam antibiotic agents, and play a strong synergistic activity against some *E. coli* strains [72].

In a study using more than 20 different bacteria strains, antimicrobial activity of TL and TB was analyzed. The TL showed at least 10-fold higher activity against all microorganisms tested than TB. In contrast with other temporins, which are known to be active against gram-positive bacteria, TL also displayed very good activity against gram-negative bacterial strains such as *E. coli* and *P. aeruginosa*. Results also showed an important cytotoxic activity to three different human tumor cell lines (Hut-78, K-562, and U-937). Another study of the interaction of TL with liposomes of different lipid compositions revealed that the peptide causes

perturbation of bilayer integrity of both neutral and negatively charged membranes. These characteristics make TL a special AMP for many of the human pathogens [98].

Another work reported the mode of action of TL on *E. coli* D21 cells. The results showed that the peptide increased the permeability of the bacterial inner membrane in a dose-dependent manner without destroying the integrity of the cell and preserving DNA integrity [99]. Also, together with TB, its mechanism of action into cell membrane has been studied in different ways, with the aim to understand its insertion into lipid monolayers as well as the effects on the structural dynamics of liposomal bilayers [100].

Finally, Saviello et al. [101] developed new analogues with potential biologic activities named Pro^3-TL and Gln^3-TA, in which Pro^3 from TA was changed by a Gln, and the Gln^3 from TL was changed by a Pro. They performed a detailed nuclear magnetic resonance analysis of the new analogues in sodium dodecyl sulfate (SDS) and dodecylphosphocholine micelles, which mimic bacterial and mammalian membranes, respectively. Their results showed that Pro^3-TL exhibited an increased antimicrobial activity toward gram-positive and yeast cells, with a hemolytic activity 2–5-fold lower than that of the natural TL, over a concentration range of 3–12 mcM. Gln^3-TA also exhibited promising antimicrobial activity, but its hemolytic activity became significantly stronger compared with that of parent peptide TA (up to 10-fold higher).

Because the hemolytic activity of TL was associated with its strong propensity for helical structure at the N-terminus, authors replaced a proline residue in position 3 with the aim of inducing a turn structure in the derivative Pro^3-TL. In the case of Gln^3-TA, according to the authors, replacement of the native proline residue with a glutamine should lead to increased helical content at the N-terminus. Their results confirmed the existence of N-terminal turn structures in the Pro^3-TL and the existence of R-helix in N-terminus region of Gln^3-TA. This confirms that helical content controls the hemolytic effect, while it is irrelevant to the antimicrobial activity of the temporins [101].

Other Temporins

Since the discovery of temporins by Simmaco et al. [10], several groups of researchers isolated different peptides from various species of frogs that were included as members of this family (Table 1). Both *S. aureus* and *E. coli* were chosen as model micro-organisms for the analysis of the antimicrobial activity against gram-positive and gram-negative bacteria, respectively. In addition, *C. albicans* was the main model organism for the analysis of the anti-micotic activity of these peptides. Table 2 resumes the antimicrobial activity of the temporins discovered until today.

Although antimicrobial activity of many temporins has not been analyzed yet, there are common properties between the peptides. For example, most of the temporins with tested antimicrobial activity have a +1 charge (Fig. 1), most temporins with antimicrobial activity against gram-negative bacteria already have activity against gram-positive bacteria, except for 1PRb from *R. pirica* [51] and the 1CS temporin family, from the skin of *R. cascadae* [29]. The two temporins without net charge (temporin GH and 1Od) have no acidic or basic amino acids and are active against gram-positive bacteria [36,48]. Those temporins with +2 net charge are only active against gram-positive bacteria, except for temporin L, CPa, and 1DRa, which are also active against gram-negative and fungi [30,31,95,102].

Some of these temporins were chosen for different antimicrobial studies. For example, temporins SHa-c (TSHa-c) was assayed against bacteria, filamentous fungi, and yeasts. The results reported by Abbassi et al. [59] showed that SHa was active against most of the tested micro-organisms at micro-molar concentrations; SHc was inactive against gram-negative bacteria but effectively inhibited the proliferation of yeasts and fungi. On the other hand, temporin TSHb was virtually inactive against all the tested micro-organisms [59].

The TSHa was also selected to design hybrid silylated biomolecules based on TSHa amphipathic helical antimicrobial sequence and titanium surfaces for the attachment of the peptides. With the design of five TSHa analogs, Masurier et al. [103] showed that the antibacterial activity of the temporin-grafted surfaces was greater on the *E. coli* strain than on the *S. epidermidis* strain, regardless of the anchoring position. Wang et al. [22] isolated a temporin family (named AL) from the skin secretion of the Chinese frog *Amolops loloensis* with antimicrobial activity against gram-positive, gram-negative bacteria and fungus, as TSHa [22].

Urban et al. [102] chose two different temporins (temporin 1DRa from *R. aurora draytonii* and temporin-1Va from *R. virgatipes*) to analyze the growth inhibitory activity of synthetic replicates of these peptides against a range of reference strains and clinical isolates of gram-positive and gram-negative [66,104]. Temporin 1DRa was the only peptide to show inhibitory activity. It also showed relatively low hemolytic activity against human erythrocytes, which could represent a potential candidate for drug development, particularly for topical therapy of infected surface lesions [102].

Conlon et al. [104] analyzed the toxicity against mammalian cells of temporin 1DRa modified by different amino acid substitutions by replacing different amino acid residues by α-aminoisobutyric acid (Aib). The Aib substitutions could induce a β-turn in small peptides but, in larger peptides, the residue could either promote the formation of an α-helix or stabilize an existing helical conformation.

This study was focused not only on the effect of these substitutions but also on the effect of the modification of the dissolution buffer. Regarding the Leu^9, Ile^{13}, Leu^{14} substitutions, the peptide presented lower cytolytic activity against mammalian cells, retaining the antimicrobial activity over the micro-organisms tested. Substitution of the Gly^4, Thr^5, Asn^8, and Ala^{10} residues by Aib produced analogs that showed increased antimicrobial potencies against the gram-positive bacteria *S. aureus* (fourfold for Aib^4 and Aib^8, twofold for Aib^5 and Aib^{10}) and against *C. albicans,* but only the Aib^8 analog showed increased potency (twofold) against the gram-negative bacteria *E. coli* [104].

Twelve analogues of temporin 1DRa were also tested as alternatives to manage infections by multi-drug resistant pathogens in skin infection. Results showed that some of these peptides were promising candidates for further development into therapeutic agents for topical treatment of skin infections. Two other temporins (temporins HN1-2, THN1-2) were tested against 12 different species of micro-organisms. Both peptides proved to be effective against all the micro-organisms tested [39,105].

Table 2. Antimicrobial and Hemolytic Activities of Temporins

Temporin	Amino acid sequence	Antimicrobial activity			Hemolytic activity	Reference
		Gram +	**Gram -**	**Fungi**		
A	FLPLIGRVLSGIL-NH_2	2.3 mcM	11.9 mcM	3.4 mcM	>120 mcM	[10]
B	LLPIVGNLLKSLL-NH_2	6.0 mcM	21 mcM	4.0 mcM	>120 mcM	[10]
L	FVQWFSKFLGRIL-NH_2	3.0 mcM	12.0 mcM	12.0 mcM	94 mcM	[10]
1ARa	FLPIVGRLISGLL-NH_2	15.0 mcM	125.0 mcM	NA	NA	[19]
1AUa	PLPIIGQLLSGLL-NH_2	>80 mcM	ND	ND	NA	[20]
ALa	FLPIVGKLLSGLSGLL-NH_2	2.0 mcM	3.0 mcM	6.5 mcM	ND	[21]
ALd	FLPIAGKLLSGLSGLL-NH_2	>1 mcM	>1 mcM	>1 mcM	7.5 μM	[22]
ALe	FFPIVGKLLFGLSGLL-NH_2	>1 mcM	>1 mcM	>1 mcM	5.8 μM	[22]
ALf	FFPIVGKLLSGLSGLL-NH_2	1.5 mcM	1.5 mcM	1.5 mcM	4.2 mcM	[22]
ALg	FFPIVGKLLFGLFGLL-NH_2	1.4 mcM	2.8 mcM	>1 mcM	8.9 mcM	[22]
ALh	FLPIVGKLLSGLSGLS-NH_2	1.6 mcM	3.1 mcM	1.6 mcM	8.1 mcM	[22]
ALi	FFPIVGKLLSGLL-NH_2	5.3 mcM	5.3 mcM	3.5 mcM	15.0 mcM	[22]
ALj	FFPIVGKLLFGLL-NH_2	5.1 mcM	10.2 mcM	3.4 mcM	13.0 mcM	[22]
ALk	FFPIVGKLLS-NH_2	13.4 mcM	26.8 mcM	13.4 mcM	22.3 mcM	[22]
1BYa	FLPIIAKVLSGLL-NH_2	15 mcM	NA	NA	NA	[23]
1Cb	FLPLFASLIGKLL-NH_2	140 mcM	NA	NA	NA	[24]
1Cd	FLPFLASLLSKVL-NH_2	80 mcM	NA	NA	NA	[24]
1Ce	FLPFLATLLSKVL-NH_2	>100 mcM	NA	NA	NA	[24]
1CEa	FVDLKKIANIINSIF-NH_2	14.4 mcM	>100 mcM	ND	NA	[25]
1CEb	ILPILSLIGGLLGK-NH_2	41 mcM	>100 mcM	ND	NA	[25]
CDYb	ILPILAPLIGGLL-NH_2	>100 mcM	>100 mcM	ND	180 mcM	[26]
CG1	FLPFVGNLLKGLL-NH_2	9 mcM	NA	NA	NA	[28]
CG2	FFPIVGKLLSGLF-NH_2	150 mcM	NA	NA	NA	[28]
CG3	FLPIVGKLLSGLF-NH_2	150 mcM	NA	NA	NA	[28]
CG4	FLPILGNLLNGLL-NH_2	NA	NA	NA	NA	[28]
CG5	FLPFVGNLLNGLL-NH_2	NA	NA	NA	NA	[28]
1CSa	FLPIVGKLLSGLL-NH_2	8 mcM	128 mcM	ND	75 mcM	[29]
1CSb	FLPIIGKLLSGLL-NH_2	8 mcM	128 mcM	ND	95 mcM	[29]
1CSc	FLPLVTGLLSGLL-NH_2	64 mcM	>128 mcM	ND	NA	[29]
1CSd	NFLGTLVNLAKKIL-NH_2	16 mcM	64 mcM	ND	50 mcM	[29]
CPa	IPPFIKKVLTTVF-NH_2	2.3 mcM	2.3 mcM	+(<20 mcM)	NA	[30]
CPb	FLPIVGRLISGIL-NH_2	ND	ND	ND	ND	[30]
1DRa	HFLGTLVNLAKKIL-NH_2	5 mcM	20 mcM	40 mcM	70 mcM	[31]
1DRb	NFLGTLVNLAKKIL-NH_2	20 mcM	40 mcM	80 mcM	65 mcM	[31]
1DYa	FIGPIISALASLFG- NH_2	>60 mcM	>60 mcM	ND	ND	[32]
1Ec	FLPIIAGLLSKLF-NH_2	8 mcM	NA	ND	ND	[33]
1Ee	FLPVIAGVLSKLF-NH_2	10 mcM	40 mcM	NA	ND	[34]
1Gb	SILPTIVSFLSKFL-NH_2	24 mcM	NA	NA	ND	[35]
1Gc	SILPTIVSFLTKFL-NH_2	25 mcM	NA	NA	ND	[35]
1Gd	FILPLIASFLSKFL-NH_2	12 mcM	NA	NA	ND	[35]
GH	FLPLLFGAISHLL-NH_2	44.3 mcM	NA	NA	ND	[36]
HN1	AILTTLANWARKFL-NH_2	37.5 mcM	NA	75 mcM	75 mcM	[39]
HN2	NILNTIINLAKKIL-NH_2	4.8 mcM	NA	9.5 mcM	NA	[39]
1La	VLPLISMALGKLL-NH_2	60 mcM	ND	ND	ND	[41]
1Lb	NFLGTLINLAKKIM-NH_2	48 mcM	NA	NA	ND	[41]
1Lc	FLPILINLIHKGLL-NH_2	125 mcM	NA	NA	ND	[41]
LK1	FFPLLFGALSSMMPKLF-NH_2	1.3 mcM	15 mcM	2.6 mcM	51 mcM	[42]
LTa	FFPLVLGALGSILPKIF-NH_2	11 mcM	ND	NA	5 mcM	[43]
LTb	FIITGLVRGLTKLF-NH_2	8 mcM	ND	NA	127 mcM	[43]
LTc	SLSRFLSFLKIVYPPAF-NH_2	25 mcM	ND	NA	100.8 mcM	[43]
LTe	FLAGLIGGLAKML-NH_2	9.5 mcM	NA	NA	40 mcM	[44]
LT1	FLPGLIAGIAKML-NH_2	9.3 mcM	ND	NA	74.5 mcM	[45]
LT2	FLPIALKALGSIFPKIL-NH_2	7 mcM	ND	NA	109 mcM	[45]
1Oa	FLPLLASLFSRLL-NH_2	2.0 mcM	70 mcM	ND	ND	[47]
1Ob	FLPLIGKILGTIL-NH_2	5 mcM	140 mcM	NA	ND	[47]
1Oc	FLPLLASLFSRLF-NH_2	2 mcM	NA	ND	ND	[47]
1Od	FLPLLASLFSGLF-NH_2	13 mcM	NA	ND	ND	[47]
1Oe	ILPLLGNLLNGLL-NH_2	NA	NA	NA	ND	[48]
1P	FLPIVGKLLSGLL-NH_2	110 mcM	ND	ND	ND	[41]
1PRa	ILPILGNLLNGLL-NH_2	>100 mcM	>100 mcM	>100 mcM	NA	[51]
1PRb	ILPILGNLLNSLL-NH_2	>100 mcM	>100 mcM	>100 mcM	NA	[51]
PRa	FLPILGNLLSGLL-NH_2	50 mcM	>100 mcM	ND	ND	[52]
PRb	FLPIITNLLGKLL-NH_2	6 mcM	>100 mcM	ND	35 mcM	[52]

(*continued*)

TABLE 2. (CONTINUED)

Temporin	Amino acid sequence	Antimicrobial activity			Hemolytic activity	Reference
		Gram +	Gram -	Fungi		
PRc	NFLDTLINLAKKFI-NH_2	50 mcM	>100 mcM	>50 mcM	80 mcM	[52]
Ra	FLKPLFNAALKLLP-NH_2	22.1 mcM	15.3 mcM	ND	ND	[54]
Rb	FLPVLAGVLSRA-NH_2	28.4 mcM	27.8 mcM	ND	ND	[54]
1Re	FLPGLLAGLL-NH_2	60 mcM	NA	NA	ND	[34]
RN1	FLPLVLGALSGILPKIL-NH_2	2.7 mcM	NA	4.7 mcM	ND	[55]
RN3	FFPLLFGALSSHLPKLF-NH_2	3.9 mcM	7.8 mcM	1.9 mcM	ND	[55]
1Sa	FLSGIVGMLGKLF-NH_2	3 mcM	10 mcM	16 mcM	25 μM	[56]
1Sb	FLPIVTNLLSGLI-NH_2	58 mcM	NA	>116 mcM	>116 μM	[56]
1Sc	FLSHIAGFLSNLF-NH_2	10 mcM	>80 mcM	20 mcM	>80 mcM	[56]
1SKa	FLPVILPVIGKLLNGIL-NH_2	25 mcM	>50 mcM	>50 mcM	ND	[57]
1SKb	FLPVILPVIGKLLSGIL-NH_2	25 mcM	>50 mcM	ND	ND	[57]
1SPb	FLSAITSLLGKLL-NH_2	6 mcM	ND	ND	60 mcM	[58]
SHa	FLSGIVGMLGKLF-NH_2	3 mcM	10 mcM	16 mcM	25 mcM	[59]
SHb	FLPIVTNLSGLL-NH_2	58 mcM	>116 mcM	>116 mcM	>116 mcM	[59]
SHc	FLSHIAGFLSNLF-NH_2	10 mcM	NA	20 mcM	>80 mcM	[59]
SHd	FLPAALAGIGGILGKLF-NH_2	6.5 mcM	>25 mcM	100 mcM	44 mcM	[59]
SHf	FFFLSRIF-NH_2	12.5 mcM	>80 mcM	50 mcM	200 mcM	[23]
SN1	FFPFLLGALGSLLPKIF-NH_2	12.5 mcM	NA	NA	ND	[61]
SN2	FITGLIGGLMKAL-NH_2	100 mcM	NA	NA	ND	[61]
SN3	FISGLIGGLMKAL-NH_2	25 mcM	NA	NA	ND	[61]
SN4	FITGLISGLMKAL-NH_2	12.5 mcM	NA	NA	ND	[61]
1Va	FLSSIGKILGNLL-NH_2	20 mcM	40 mcM	NA	120 mcM	[66]
1Vb	FLSIIAKVLGSLF-NH_2	10 mcM	NA	NA	30 mcM	[66]
1Vc	FLPLVTMLLGKLF-NH_2	10 mcM	NA	NA	30 mcM	[66]

The criteria were selected according to the bibliography: High (minimum inhibitory concentrations [MICs] <5 mcM); positive MICs <50 mcM); low (MICs between 50 mcM and 150 mcM); NA (not active up to 150 mcM); ND (no data). In all cases, the micro-organisms selected for the information were *Staphylococcus aureus*, *Escherichia coli,* and *Candida albicans*.

Temporin CPa from *Lithobates capito* has an atypical sequence IPPFIKKVLTTVF-NH_2, and unlike most of the temporins, it showed atypical growth-inhibitory activity having greater potency against *E. coli* and *C. albicans* than against *S. aureus* [30]. Yang et al. [28] found a group of peptides (temporins TC 1-5, TCG_{1-5}) with antimicrobial activity against gram-positive bacteria at low concentrations compared to other AMPs. Abbassi et al. [56] isolated three peptides, named temporins 1Sa-c from the skin of the Sahara frog *Pelophylax (Rana) saharica*. As temporin 1G, 1Sa was effective against gram-positivebacteria [56].

Some temporins have low or no antimicrobial activity, although they present conserved features to the active peptides (13 amino acids, one basic amino acid, +1 charge). For example, temporins SPa-c showed very low antimicrobial activity, with positive response only for *S. aureus* [58]. Temporins 1PRa-b, Ska-b, and LT also showed low antimicrobial activity with gram-positive bacteria, very low or no activity with gram-negative bacteria, and no effectiveness against yeasts [35–37,41,43,51,57,62,105,106].

Finally, new temporins were found in different researches. The first, temporin Wa (TWa) was found in *Lithobates warszewitschii*, and it was not possible to establish the complete amino acid sequence (FISKIASLGAGVLX-NH_2, Table 1). A second temporin found in the skin secretion of *R. muscosa* (1M) was isolated as free acid form. This was an unexpected finding because the presence of such peptides in frog skin secretions has not been reported previously. Authors determined that it is unclear whether the peptide is an authentic secretion product or is derived by artifactual hydrolysis during the extraction and purification process. Experiments with a pathogenic chytrid fungus showed that this temporin inhibited chytrids at concentrations above 6.25 mM with a minimum inhibitory concentration (MIC) of 100 mM against zoospores [46]. No other antimicrobial activity has been tested yet.

A third one, temporin LK1 (TLK1), was found in *Limnonectes kuhlii* frogs. This peptide of 17 amino acids showed antimicrobial activity against gram-positive and gram-negative bacteria and fungi [42]. This temporin was also selected for an antimicrobial activity analysis of five structural analogs. These peptides were prepared by substitution of achiral glycine residue of TLK1 with D-alanine, L-phenylglycine, and L-naphthylalanine, respectively. Analogs 2–4 showed activity against *P. aeruginosa* and *S. aureus* [106].

Pharmacologic Application

Antibiotic agents are essential in medicine for the control of pathogens in human and other species. Their abuse and misuse, however, have increased the number of resistant micro-organisms. This entails the need to develop new and better alternatives for multi-drug–resistant bacteria [107,108]. Peptides are molecules with a simple chemical structure that can be synthesized at low-cost on a large scale. In addition, it is feasible to chemically modify them to improve their bioavailability, pharmacokinetics, and pharmacodynamics, among other advantages.

These properties make them good candidates for new drug developments, especially those with synergistic effect when more than one peptide is combined. This can be exemplified by the combinations of TA, TB, and TL in studies against *E. coli* strains. As a consequence of the antibiotic therapy of gram-negative bacterial infections, LPS or endotoxins can be released. The use of compounds capable of sequestering LPS has been suggested to avoid sepsis. These three temporins showed a synergistic effect on LPS sequester [109,110]. On the other hand, TL avoided the LPS-induced self-assembly of TA and TB. For this reason, it has been suggested that AMPs in combination with other drugs, could be good candidates for antibacterial or anti-endotoxin treatment, avoiding the generation of bacterial resistance to the therapy [96,111,112]. These temporins could be used as potential drugs and represent promising alternatives to supply antibiotic agents.

In addition, temporins B and L can penetrate lipid monolayers and generate molecular interaction with different liposomes, which could be an alternative through the encapsulation of these peptides for drug administration [100]. Temporin 1DRa also could represent a potential candidate for a drug, as was explained earlier [102,105,113].

Today, the new challenge is to load the peptides into polymeric drug delivery systems. Recently, Piotrowska et al. [81] developed new polymeric carriers to control the release of TA and other AMPs to improve the use of new therapeutic candidates for the management of local infections.

Antifungal resistance has become a challenge for new drug design. Currently there are only five therapeutic alternatives for the management of fungal infections (azoles, echinocandins, polyenes, allylamines, and pyrimidine analogs). Most antifungal agents show high toxicity and undesirable side effects that limit their use in medicine. Patients with immunodeficiencies are more likely to have fungal infections develop. The use of antifungal agents as prophylactic agents, however, has favored the emergence of drug resistances in species of *Candida* and *Cryptococcus* as well as other pathogenic fungi [113,114].

In addition to the design strategies for the production of more efficient drugs against pathogens with antibiotic resistance, the use of nanotechnology has been proposed for the development of new administration systems that are expected to spread widely and rapidly. In this way, the reduction of toxicity and side effects of current dugs can be achieved [115]. Combination of nanotechnology and AMPs in different antimicrobial treatments could be a promising alternative for resistance pathogens. Therefore, drugs must be of low cost so that lower income groups can easily get a treatment with new novel drug molecules, which can help to fight against drug resistance [108].

Conclusion

Although temporins have been gaining importance in antimicrobial studies, many of them have not been fully analyzed yet. Most of these peptides have shown interesting and promising properties, together with the advantages explained in this article. This makes this family a potential alternative for pharmacology and biotechnology industries, for the research of new drugs with more effective administration methods and minor injuries.

Although recent researches with the most studied temporins (TA, TB, and TL) showed the potential of these small peptides, the temporin family is still an unexplored AMP group in pharmacology industries. Some recently discovered temporins do not present photosensitive amino acids (as cysteine or tryptophan), which is an advantage for drug delivery, because the molecular and chemical stability of the peptides allow an efficient and secure administration. Although TL showed good antimicrobial activity, it has lower stability than other temporins, because it presents a tryptophan in its sequence.

Initially, TL was the only temporin with anti–gram-negative activity, but today there are many other peptides with the same antimicrobial activities and are more stable. These studies demonstrate that, even with the advancement in scientific research on the composition and antimicrobial activity of temporins, further studies are necessary to wholly understand their components and mechanisms of action.

The need for new and more effective antibiotic and antifungal agents makes necessary the discovery and application of new drugs based on natural peptides such as AMPs.

Future Prospectives

The AMPs are the future drugs and the promising alternative in the pharmacologic industry. Combination of AMPs with nanotechnology could be the answer to all the resistant pathogens that produce thousands of mortal diseases per year, especially in poor and underdeveloped countries. New discoveries in AMPs activities and efficiency are necessary to incorporate more suitable alternatives in the field of medicines. Temporins are a promising candidate for the improvement of new drugs doe topical use and oral administration. This is the next step in the investigation of these peptides that are not deeply studied.

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