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ANTIMICROBIAL PEPTIDES AS PARASITICIDAL AGAINST HUMAN TRYPANOSOMATIDS: MECHANISMS OF ACTION AND CURRENT STATUS IN DEVELOPMENT

By

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Abstract

Trypanosomes cause a variety of tropical diseases that affect the livelihood of individuals worldwide. The currently used pharmaceutical treatments rely on chemotherapy. However, many of these drugs are very expensive, and highly toxic. In addition, parasite resistance to several of the therapeutic drugs used is increasing. Therefore, there is a growing need for new control measures for many of these diseases. One new approach is the use of antimicrobial peptides (AMPs) to disease control, since these peptides can be used as potential anti-parasite effector molecules. This review summarizes and discusses the parasiticidal properties of AMPs for treating trypanosome infections, highlighting their mechanisms of action and current status in development.

Key words: Antimicrobial peptides, *Leishmania, Trypanosoma,*human infection.

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Introduction

Parasitic diseases caused by trypanosomatid parasites *Leishmania, Trypanosoma cruzi* and *T. brucei* remain an unsolved public health problem that affects millions of people worldwide (Desjeux, 2004). About 12 millions are infected with *Leishmania* http: //www. who.int/leishmanisis/burden/magnitude /en/index.html). So, there is a clear need for the development of new therapeutic agents against these parasites. At present, the effectors with anti-parasite potential are the antimicrobial peptides (AMPs), integral molecules of innate immunity (Méndez-Samperio, 2008; Méndez-Samperio, 2010). The first isolated AMPs were the cecropins, from hemolymph of the moth *Hyalophora cecropia* (Hultmark, 1980). An impressive number of more than 1500 AMPs were listed in different databases (Fjell *et al*, 2007, Wang *et al*, 2009). Although great sequence diversity exists, the majority of AMPs contain relative high percentages of basic amino acids and are classified based on secondary structural features, such as cathelicidins (with a linear α-helical st-

ructure) or defensins (with a β-strand structure) (Boman, 2003; Ganz and Lehrer, 1995; Wang and Wang, 2004). The main role of antimicrobial peptides is direct lysis of microorganisms via the electrostatic interaction with the cell target, leading to membrane disruption (Lehrer *et al*, 1993, Oren and Shai, 1998). AMPs cross the microbial membrane and associate with intracellular targets such as DNA, DnaK chaperone and mitochondria (Luque-Ortega *et al*, 2008). The discovery of the magainins in the skin of *Xenopus laevis* (Zasloff, 1987) and cecropins represented a promising route to develop new antiparasitic drugs either to treat infected hosts, or to prevent disease transmission by interfering with their insect vectors (Gwadzt *al,* 1989; Boman *et al*, 1989).

Mammalian infection occurs by the delivery of parasites into the skin of infected insects. The susceptibility to parasite infection results from the expansion of a TH2 response leading to production of IL-4 and IL-10 (Reiner *et al*, 1993; Reiner and Locksley 1995). It has been demonstrated that susceptibility of BALB/c mice to infection with *Leishmania mexicana* and *L. amazonnsis* was dependent on IL-10 production (Padigel *et al*, 2003). Moreover, IL-10 may control the secretion of effector molecules such as nitric oxide (NO) in infected macrophages and affect the elimination of intracellular parasites (Kane and Mosser, 2001). The intracellular amastigote stage of *Leishmania* resides within phagolysosome of macrophages, while intracellular trypanosomes can reside in any nucleated cell type. In a mouse model of cutaneous leishmaniasis, resistance to infection was dependent on a TH1 response. T cells produced interleukin (IL)-12 and (interferon) IFN-γto control the later stages of parasitic infection. The control of visceral leishmaniasis of humans was dependent on a TH1 response (Murray, 1997; 2005).

AMPs are expressed with elements of immunity and its secretion might be constitutive or inducible. Expression of defensins was upregulated in human cells exposed to IFN-γ (Fang *et al*, 2003). Because in immune response of insect vectors AMPs are important components of innate immunity, after infection with parasites several AMPs were detected locally in the gut of insect-vectors (Hao *et al*, 2001; Boulanger *et al*, 2002).

Lopez *et al*. (2003) found that upon infection with *T. cruzi* a defensin produced in the fat body of the insect vector. The kinetics of AMP induction following trypanosomatid parasites ingestion varies according to parasite stage development. In fact, ability of insect vector to recognize surface antigens of trypanosomatid parasites determined the induction of AMP synthesis (Haines *et al*, 2003).

The present authors reviewed and discussed efforts to determine the antiparasitic properties of AMPs for treating trypanosome infections, highlight some of the mechanisms by which AMPs control different stages of these parasites, and summarized the potential use as an alternative to treating leishmaniasis and/or trypanosomiasis.

Review and General Discussion

The antiparasitic activities of AMPs against *Leishmania* and *Trypanosoma* species**:** Several AMPs have direct antiparasitic activity against *Leishmania*. For example, it was reported that the leishmanicidal activity of Hyalophora cecropin A (Akuffo *et al,* 1998). It was shown that cecropin A (1-8)-melittin (1-18) (CAMEL) hybrid peptides caused morphological damage of the plasma membrane of *Leishmania donovani* promastigotes followed by rapid loss of ATP (Diaz-Achirica *et al*,1998). CAMEL analogues (11-15 residues) shown to have a more leishmanicidal activity than theparental peptide (Luque-Ortega *et al*, 2003). In this regard, N-terminal fatty acid substitution increases the lethal effect of CA(1-7) M (2-9), a cecropin–melittin hybrid peptide against *L. donovani* promastigotes and amastigotes (Chicharro *et al*, 2001). A clinical study, demonstrated the efficacy of N-terminal Octyl-CA (1-7) M (2-9) against naturally acquired leishmaniasis in infected dogs (Alberola *et al*, 2004). In support to leishmanicidal activities of AMPs, it was shown that gomesin, and 18-residue cysteine-rich peptide from spider *Acanthoscurriagomesiana* hemocytes was a potent AMP against *L. amazonensis* promastigotes (Silva *et al*, 2000). Besides, a defensin from sand fly *Ph. duboscqi* has an important antiparasitic activity against *L. major* promastigotes (Boulanger *et al*, 2004).

Nowadays, a broad range of mammalian AMPs have activity against *Leishmania*, inducing cell death by different mechanisms. The various mammals cathelicidins (protegrin-1 [porcine], SMAP-18 and myeloid AMP-27 [bovine]) and defensins have an important leishmanicidal activity on promastigotes of *L. major* and *L. amazonensis* by inducing apoptosis (Kulkarni *et al*, 2006). Also, indolicidin a bovine neutrophil AMP diminished motility of *L. donovani* promastigotes by inducing breakdown of membrane potential and caused autophagy-like parasite death (Bera *et al*, 2003). Of interest, Luque-Ortega *et al*. (2008) showed that histatin 5, a human salivary AMP was parasiticidal against *L. donovani* the promastigotes and the amastigotes by inducing a significant reduction of ATP activity ending in cellular death, indicating this molecule is a true cell-penetrating peptide. Kulkarni *et al.* (2009) reported that AMP-mediated apoptosis death of *Leishmania* involved massive delocalization of intracellular calcium.

Regarding synthetic AMPs, an analogue of magainin, called pexiganan was used to demonstrate importance of positional hydrophobicity in parasiticidal effect on *L. major* (Guerrero *et al*, 2004). Besides, it was showed that this synthetic AMP has antiparasitic effects through the peptide-induced apoptotic killing; a process resulted from calcium dependent and caspase-independent mitochondrial toxicity (Kulkarni *et al*, 2006). It has been acknowledged that the 8-amino-quinoline analogue sitamaquine is an AMP causing an important inhibition of complex II of the respiratory chain in the digitonin-permeabilized promastigotes, together with a drop in intracellular ATP levels and a decrease of the mitochondrial

electrochemical potential, leading to an apoptosis-like death of *L. donovani* (Carvalho *et al*, 2011).

Of the AMPs isolated from aquatic animals cyclized-defensins and cysteine-rich mytilin A (from mussels) have anti-parasite activities against *L. major* (Roch *et al*, 2004). Studies demonstrated that tachyplesins (from the horseshoe crab) are the most potent peptides against trypomastigotes of *L. braziliensis* and *T. cruzi* (Lofgren *et al*, 2008). Besides, dermaseptins, AMPs isolated from genera *Phyllomedusa* have a potent leishmanicidal activity inducing structural damage in *L. mexicana* (Hernandez *et al,* 1992) and *L. major* (Savoia *et al*, 2008).The anti-leishmanial activities against *L. amazonensis* promastigotes of phylloseptin-1, an AMP isolated from related frog *Phy. azurea* (Kuckelhaus *et al,* 2009). The AMPs temporin A &B, isolated from old and new world tree frogs, have significant activity against promastigotes of *L. donovani* (Mangoni *et al*, 2005). These authors have demonstrated that the main lethal effects of these AMPs are through surface-membrane permeability changes and loss of intracellular ATP. Besides, Mangoni *et al*. (2006) reported that bombinin-H4 is the first native AMP with a D-alloisoleucine amino acid, isolated from the skin of frog, which leads to higher permeabilization against both promastigotes and amastigotes from *L. donovani*. This work of Rivas lab showed the activities of different AMPs on the cellular membrane of *Leishmania* inducing membrane permeabilization led to parasite death (Diaz-Achirica *et al,* 1998; Guerrero *et al,* 2004; Mangoni *et al,*2005). But, these authors sometimes used concentrations far in excess of what the parasites would encounter under physiological conditions. Abbassi *et al*. (2008) found that temporin-1Sa, a new AMP isolated from the skin of North African ranid *Pelophylax saharica* has important antiparasitic activities against *L. infantum.*Abbassi *et al.* (2010) reported a new type of AMP designated temporin-SHf with a short length, compositional simplicity, and broad-spectrum activity (Tab. 1)

Also, the wheat thionins were AMPs isolated form plants with leishmanicidal activity against *L. donovani* promastigotes by disrupting the cell membrane (Berrocal-Lobo *et al,* 2009).

AMPs were found capable to control *Trypanosoma* species development. Jaynes *et al*. (1988) showed that *T. cruzi* killed by two lytic peptides (SB-37 & Shiva-1*) in vitro*. Barr *et al,* (1995) *in vitro* study, reported that synthetic homologues of cecropin B activity of lytic peptides against *T. cruzi* trypomastigotes.Also, Beard *et al.* (2002) reported that cecropin-A expressed paratransgenically within *Rhodnius prolixus* (a vector of *T. cruzi*) reduced the number of infective metacyclic trypomastigotes within the insect GI tract. Hu and Aksoy (2005) found that attacin was an AMP isolated from tsetse fly with a potent trypanocidal activity against *T. brucei*. Furthermore, trialysin an AMP from saliva of hematophagous insects had a significant lethal effect on *Trypanosoma* species (Amino *et al,* 2002; Martins *et al,* 2006; Martins *et al*, 2008). A lethal

effect on trypanosomes was shown in several beetle defensins. Specifically, Kitani *et al*. (2009) found that synthetic nonamer peptides derived from insect defensin mediate the killing of African trypanosomes. AMPs obtained from aquatic animals have been reported to be active against *T. cruzi* amastigotes. Dermaseptins were highly active at as low as 0.4 M without cytotoxicity to mammalian cells (Brand *et al,* 2002). AMP, DShypo-01 isolated from the *Phy. hypochondrialis* was more active against trypanosomes (Brand *et al,* 2006).

Mammalian cathelicidins: BMAP-27, indolicidin, LL-37, SMAP-29, SMAP analogues: ovispirin, novispirin, and protegrin-1 had an important anti-parasitic activity against trypanosomes (McGwire *et al*, 2003). The pretreatment of trypomastigotes with bovine homologue of the human membranolytic peptide granulolysin (NK-lysin) and shortened analog NK-2 reduced trypomastigotes capacity to establish intracellular infection in host cells (Jacobs *et al,* 2003). Human vaso-active-intestinal-polypeptide was an active AMP killed *Trypanosoma* spp. (Gonzalez-Rey *et al,* 2006). Haines *et al.* (2009) reported that BMAP-18 was also an active AMP against trypanosomatid parasites.

Mechanisms of action and signaling pathways for antiparasitic AMPs: Antiparasitic activities of AMPs occurred by a number of different mechanisms. In all mechanisms of action of AMPs it is important to consider the structural features of these molecules, such as cationic character and amphipathycity (Epand and Epand, 2009; Thennarasu *et al,* 2010; Palermo *et al,* 2011). Primary structure of AMPs with parasiticidal action against pathogenic trypanosomatidsare was given (Tab. 2).

This structure-activity relationship is referred to the capacity of AMPs to induce membrane permeabilization by distortion of the parasite membrane structure, inducing a killing mechanism (Hwang and Vogel, 1998; Hancock and Rozek, 2002). In this regard, the importance of two amino acids (Arg & Trp) in the structure of different AMPs was established. Arg residues endow the peptides with cationic charges and hydrogen bonding properties necessary for interaction with the abundant anionic components of parasite membrane, while Trp has a distinct preference for the interfacial region of lipid bilayers. It is important to consider that this mechanism of action occurs in a few minutes (Feder *et al,* 2000), and it does not need to interact with any receptor. At present, exists various models of AMP activity to induce membrane permeabilization, and in these models the AMP specificity of action relies on the composition of parasite membrane. In all models, the interaction with the outer and /or inner membranes of parasite is necessary (Chan *et al,* 2006). In the carpet model, the peptides are lining up parallel to the membrane surface, forming a peptide carpet and is bases on the amphipathic character of the AMPs (Papo and Shai, 2003; Huang *et al,* 2004). In the barrel-stave model, the peptides span the membrane inducing a pore on the parasite membrane and occurred at very low peptide:

phospholipid ratio (Shai, 2002). In the two-state model based on studies of representative cysteine-rich AMPs, the unionof AMPs parallel to the plane of the parasite membrane led to its expansion, inducing a mechanical stress. In this model, the binding of the amphipathic peptides causes an increase in local membrane curvature, forming transient pores. Once the peptides continue assembling on the membrane surface and exceed the peptide/lipid ratio threshold, the peptide assemblages becomes perpendicular to the plane of membrane and form trans-membrane pores (Jang *et al,* 2006; Huang, 2006). In the aggregate model, AMPs create pores that contain peptides as well lipid molecules that are curve to adopt a prototypical cylindrical shape. In the Droste mechanism the toroidal lumen adopts a poor orientation provided by the curvature of the phospholipids (Sengupta *et al,* 2008).

The current literature indicates that in early parasitic infection, parasites have mechanisms to neutralize innate immune components and AMPs (Brittingham *et al,* 1995). It has been reported that *Leishmania* have resistance strategies to mechanism of action of AMPs, such as proteinase production (Croft *et al,* 2006; Delespaux and Koning, 2007). The very fast kinetics of the mechanism of action reduced the possibility of this resistance strategy. Another parasite resistance mechanism relies on the suitability to intracellular parasitism. However, several AMPs proved to be very active against intracellular parasites (Mangoni *et al,* 2006; Savoia *et al,* 2008; Rivas *et al,* 2009).

An alternative mechanism of action of antiparasitic AMPs is through intracellular signaling mechanisms initiated with the parasite membrane disruption (Otvos, 2005). The AMPs with leishmanicidal activities caused important changes in ATP content led to parasite death (Luque-Ortega *et al,* 2001; 2003). The subcellular structures affected by antiparasitic AMPs, mechanism of action of the mammal AMP histatin 5 was by inducing a decrease of mitochondrial ATP synthesis leading to *Leishmania* death (Luque-Ortega *et al,* 2008). Thus, different organelles for intracellular calcium storage in protozoan parasites were important targets for different mechanisms of AMPs action (Moreno and Docampo, 2003). The mechanism of action of different AMPs was though a calcium dependent signaling pathway to *Leishmania* death (Kulkarni *et al,* 2009). Another mechanism of action of antiparasitic peptides was by inducing apoptosis and necrosis (Kulkarni *et al,* 2006; 2009). They reported that AMP-induced apoptosis led to an important delocalization of intracellular calcium which induced a caspase-independent mitochondrial toxicity. It was reported citotoxic effect of a mammalian AMP of defensin-class on *T. cruzi* by inducing DNA fragmentation (Madison *et al,* 2007). Antiparasitic effect of AMPs resulted from both its direct antiparasitic activity and indirect immune regulatory functions.

Several AMPs have exerted chemoattractant activity for immune cells (Rivas *et al,* 2009). A list of general action mechanisms and signaling pathways

for antiparasites activities of natural and synthetic AMPs against pathogenic trypanosomatids presented (Tab. 3). Several vertebrates evolved different mechanisms to target their AMPs to parasites while limiting damage to themselves, including controlled synthesis of mature AMPs, and storage of AMPs in cell compartments such as neutrophil granules (Murakami *et al,* 2004; Lehrer, 2007).

AMP-based drug development: several antiparasitic therapeutic drugs are prohibitively expensive to patients with trypanosomiasis and have unacceptable toxicity. There is increasing drug resistance in target populations of parasites, due to endocytosis and exocytosis processes are restricted to flagellar pocket and that the plasma membrane structure of promastigote form possesses a thick anionic glycocalix made up mostly of lipophosphoglycan. The AMPs have the advantages over traditional drugs, since AMPs naturally occupy positions in immunity inducing pleiotropic activities in controlling the parasites. Previous studies have discussed AMP-based drug development (Andre`s and Dimarcq, 2005; Hancock and Sahl, 2006). Most of these studies are in the discovery or preclinical stages with some proceeding to clinical trials. Nisin is one example of AMP-based antibiotic therapies that have been commercialized. Other AMP-based drugs that have progressed to clinical trials are the derived from insect cecropin B and bovine indolicidin (Hancock and Sahl, 2006; Scott *et al,* 2007). These AMPs were developed to treat skin-related infections in humans. Some drugs are derivatives of AMPs that have been modified introducing non-natural residues like D-amino acids or addition of C-terminal amidation to improve their antimicrobial activity (Bansal *et al,* 2008).

It should be considered in developing AMP-based drugs, that an ideal antiparasite agent must have a broad spectrum of activity, low toxicity for the recipient, and a low propensity to induce parasite resistance. Another important challenge to the AMP-based drug development in trypanosomiasis is the higher cost of peptide synthesis. Also, from a therapeutic perspective in trypanosomiasis the important data required for a given AMP are its parasiticidal activities and therapeutic index.

Thus, it is important to consider that antiparasitic activities of AMPs are defined by membrane permeation activity. Since most of the antiparasitic drugs used have low efficiency of drug targeting (Kayser and Kiderlen, 2003), the discovery of AMPs as new drugs relies on the identification of new targets to induce structural parasite damage. Ordonez-Gutierrez *et al*. (2007) have reported the effect of new formulations of amphotericin-B on amastigote and promastigote forms of *L. infantum*, indicating the use of microemulsions, micro-particles or nanoparticles as new formulation approaches. Also, BMAP-18 proved a good candidate for testing *in vivo* as a drug for hosts (Haines *et al,* 2009).

Table 1: AMPs isolated from aquatic animals with parasiticidal effects against trypanosomatid parasites:

AMPs	µM	Parasiticidal effect	Parasite species	stage
Bombinin-H2[1]	7.3	Inhibition of cell proliferation	*L. donovani*	promastigote
Bombinin-H4[1]	1.7	Intracellular parasite killing	*L. donovani*	promastigote/amastigote
Dermaseptin-1[2]	5.6	Intracellular parasite killing	*L. pifanoi*	Amastigote
	2.8	Citocidal effect	*T. cruzi*	trypomastigote
Dermaseptin-1[3]	11.6	Citocidal effect	*L. amazonensis*	promastigote
Dermaseptin-D11[2]	2.5	Parasite death	*T. cruzi*	trypomastigote
Dermaseptin-D11[2]	2.8	Parasite death	*L. major*	promastigote
Dermaseptin-H3[3]	13.5	Inhibition of cell proliferation	*L. amazonensis*	promastigote
Dermaseptin-S1[4]	2.3	Intracellular parasite killing	*L. major*	promastigote/amastigote
Dermaseptin-S1[8]	1.5	Inhibition of cell proliferation	*L. mexicana*	promastigote
Dermaseptin-S4[6]	1.5-2	Inhibition of cell proliferation	*L. major*	promastigote
Magainin-2[7]	0.9	Parasite death	*L. major*	promastigote
Mylitin A[8]	8.0	Parasite death	*L. major*	promastigote
Phylloseptin-1[9]	5.1	Inhibition of cell proliferation	*L. amazonensis*	promastigote
Tachyplesin[10]	100.0	Parasite death	*T. cruzi*	trypomastigote
Tachyplesin[10]	100.0	Parasite death	*L. braziliensis*	promastigote
Temporin A[11]	8.4	Killing of promastigotes	*L. donovani*	promastigote
Temporin B[11]	8.6	Killing of promastigotes	*L. donovani*	promastigote
Temporin-1Sa[12]	18.1	Inhibition of cell proliferation	*L. infantum*	promastigote

[1]Mangoni*et al*, 2006; [2]Brand *et al*, 2002; [3]Brand *et al*, 2006; [4]Savoia *et al*, 2008; [5]Hernandez *et al*, 1992; [6]Feder *et al*, 2000; [7]Guerrero *et al*, 2004; [8]Roch *et al*, 2004; [9]Kuckelhaus *et al*, 2009; [10]Lofgren *et al*, 2008; [11]Mangoni *et al*, 2005; [12]Abbassi *et al*, 2008.

Table 2: Primary structure of AMPs with parasiticidal effects against trypanosomatid parasites

AMPs	Primary structure	Parasite (stage)	References
Bombinin-H2	IIGPVLGLVGSALGGLLKKI	*L. donovani* (pro)	Mangoni*et al*, 2006
Bombinin-H4	IIGPVLGLVGSALGGLLKKI	*L. donovani* (pro)	Mangoni*et al*, 2006
α-Defensin	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	*T. cruzi* (try)	Madison *et al*, 2007
0-11 Defensin	Cyclic GVCRCLCRRGVCRCLCRR	*L. amazonensis* (pro)	Kulkarni *et al* 2006
Dermaseptin-1	GLWSTIKQKGKEAAIAAAKAAGQAALGAL	*T. cruzi* (try)	Brand *et al*, 2002
		L. amazonensis (pro)	Brand *et al*, 2006
Histatin 5	DSHAKRHHGYKRKFHEKHHSHRGY	*L. donovani* (pro)	Gwadz *et al*, 1989
Magainin-2	GIGKFLHSAKKFGKAFVGEIMNS	*T. pyriformis* (try)	Soravia *et al*, 1988
NK-2	KILRGVCKKIMRTFLRRISKDILTGKK	*T. cruzi* (try)	Jacobs *et al*, 2003
Tachyplesin	KWCFRVCYRGICYRRC	*L. braziliensis* (pro)	Lofgren *et al*, 2008
		T. cruzi (try)	Lofgren *et al*, 2008
Temporin A	FLPLIGRVLSGIL	*L. donovani* (pro)	Mangoni *et al*, 2005

Abbreviations: ama, amastigote; pro, promastigote; try, trypomastigote.

Conclusion

There is enough evidence that some AMPs exhibit potent killing activity *in vitro* against trypanosomatid infections. For therapeutic applications *in vivo*, there are ongoing concerns about AMP toxicity, serum sensitivity and stability of the AMPs in the treated animals' blood. New fields of antiparasitic research are the methods helpful in defining the antiparasitic mechanisms directly to the target parasite. These studies could assist in development of new AMP-based drugs as strategy for trypanosomatid infections. Future studies might build and exploit on the multifunctional properties of new AMP-based drugs as antiparasitic effectors validating *in vivo* AMPs functions that protect against trypanosomatids. It is evident that optimized design of AMPs based on knowledge from natural AMP

studies provides promising alternatives to present therapy of trypanosomiasis

Table 3: Mechanisms of action and signaling pathways for antiparasitic activities of natural and synthetic AMPs against pathogenic trypanosomatids

Origin:	Antiparasitic activities	Mechanisms of action/signaling pathways
AMPs		
Aquatic animals		
Bombinin-H4[1]	Leishmanicidal activity	Membrane permeabilization & chemotaxis
Dermaseptin-1(2)	Leishmanicidal activity	Membrane disruption leading to structural damage
Magainin-2 [3]	Leishmanicidal activity	Apoptotic killing; ca^{2+}-dependent signaling pathway & caspase-independent mitochondrial toxicity
Tachyplesin [4]	Leishmanicidal&trypanomicidal activities	Membrane permeabilization and bind to DNA
Temporin A[5]	Leishmanicidal activity	A ca^{2+}-dependent signaling pathway leading to parasite death
Temporin B[6]	Leishmanicidal activity	Surface-membrane permeability changes & loss of intracellular ATP
Insect:		
Attacin[7]	Trypanocidal activity	Membrane disruption
Cecropin A[8]	Trypanocidal activity	Reduce the number of infective metacyclic trypomastigotes
Cecropin B[9]	Leishmanicidal activity	Reduction in ATP content leading to membrane disruption
Melittin[10]	Leishmanicidal activity	Membrane disruption and chemotaxis
Trialysin[11]	Trypanocidal activity	Membrane disruption by a pore-forming protein
Mammals:		
α-Defensin[12]	Trypanocidal activity	Induce to DNA fragmentation leading to necrosis
Histatin 5[13]	Leishmanicidal activity	Decrease of mitochondrial ATP synthesis & breakdown in mitochondrial membrane leading to parasite death
Indolicidin[14]	Leishmanicidal activity	Breakdown of membrane potential; autophagic-like parasite death
Myeloid AMP-18[3]	Leishmanicidal activity	Apoptotic killing
Plants:		
Wheat thionin[15]	Leishmanicidal activity	Membrane disruption
Synthetic peptides		
CA(1-8)M(1-18)[16]	Leishmanicidal activity	Morphological damage of plasma membrane
Sitamaquine [17]	Leishmanicidal activity	Inhibition of complex II of respiratory chain; a drop in intracellular ATP levels; decrease in mitochondrial electrochemical potential

[1]Mangoni *et al*, 2006;[2]Brand et al, 2006; [3]Kulkarni et al, 2006; [4]Lofgren et al, 2008; [5]Kulkarni et al, 2009; [6]Mangoni et al, 2005; [7]Hu & Aksoy, 2005; [8]Beard et al, 2002; [9]Luque-Ortega et al, 2003; [10]Luque-Ortega et al, 2001; [11]Martins et al, 2008; [12]Madison et al, 2007; [13]Luque-Ortega et al, 2008; [14]Bera et al, 2003; [15]Berrocal-Lobo et al, 2009; [16]Diaz-Achirica et al, 1998; [17]Carvalho et al, 2011.

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