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Review

Antimicrobial peptides as an alternative to anti-tuberculosis drugs

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ABSTRACT

Tuberculosis (TB) presently accounts for high global mortality and morbidity rates, despite the introduction four decades ago of the affordable and efficient four-drugs (isoniazid, rifampicin, pyrazinamide and ethambutol). Thus, a strong need exists for new drugs with special structures and uncommon modes of action to effectively overcome *M. tuberculosis*. Within this scope, antimicrobial peptides (AMPs), which are small, cationic and amphipathic peptides that comprise a section of the innate immune system, are currently the leading potential agents for the treatment of TB. Many studies have recently illustrated the capability of anti-mycobacterial peptides to disrupt the normal mycobacterial cell wall function through various modes, thereby interacting with the intracellular targets, as well as encompassing nucleic acids, enzymes and organelles. This review presents a wide array of antimicrobial activities, alongside the associated properties of the AMPs that could be utilized as potential agents in therapeutic tactics for TB treatment.

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1. Introduction

Human tuberculosis (TB) is the manifestation of the infection of humans by members of the *Mycobacterium tuberculosis* complex, comprising *Mycobacterium tuberculosis*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium microti*, *Mycobacterium leprae* and *Mycobacterium canetti* [1]. The patients suffering from active pulmonary TB are considered as the main source of infection, while most people carrying the *M. tuberculosis* infection remain asymptomatic (latent TB infection (LTBI)). Globally, there are about two billion LTBI cases, who are in danger of the disease being reactivated [2,3]. Despite the introduction of the aforementioned four affordable and efficient drug treatment regimens four decades ago, TB has continued to spread to all corners of the world [4].

The World Health Organization's (WHO) 17th report on the global occurrence of TB signifies that it maintains the status of a global emergency. It was estimated that in 2015, there were about 10.4 million new cases and 1.8 million deaths from TB, including 0.4 million deaths among people co-infected with HIV. The greatest rates per capita of TB are found in sub-Saharan Africa, which is mainly challenged by the HIV epidemic in the region. Nearly 60% of global TB cases are found in South Africa, China, India and the Russian Federation [5]. In the United States and Western Europe, the majority of TB cases are found among residents from other nations where TB is highly endemic [6,7]. Currently, multidrug-resistant TB (MDR-TB) is widespread, with around 580,000 documented cases for 2015. Extensively drug-resistant TB (XDR-TB) has currently been reported in about 84 countries of the world. Owing to the financial limitations and laboratory infrastructural inadequacy, only about 19% of the globally approximated numbers of MDR-TB cases were reported to the WHO in 2011, while less than 4% of MDR-TB cases are presently diagnosed at a global level. The treatment of TB is challenging, requiring a precise diagnosis, screening for resistance to drugs and the administration of effective treatment regimens for a minimum of six months through directly observed therapy (DOT). Therefore, there is a need for the development of novel TB agents for shorter TB treatment regimens [8].

The study of AMPs commenced during World War II when gramicidin, an antimicrobial agent from the soil bacterium *Bacillus brevis,* was discovered by Rene J. Dubois. This antimicrobial agent was found to be effective against Gram-positive bacteria and used for the treatment of soldiers' wounds and ulcers [9]. Nonetheless, AMP as a term was not broadly used before the 1960s when a number of proteins with selective antimicrobial actions in polymorphonuclear (PMN) leukocytes were discovered [10–12]. During the 1980s, cecropins were isolated from larva, followed by the unveiling of magainins from the skin of frogs, indicating that AMPs are made in the higher vertebrates [13]. Two mammalian AMPs were identified in the 1980s, comprising cathelicidins and defensins, albeit with several scrutinies. Defensins can be categorized into α-defensins, β-defensins and θ-defensins [14]. Described as cationic peptides, cathelicidins have a common N-terminal cathelin-like domain, but possess a variable C-terminal region.

The defensins, as well as the cathelicidins, have a wide and varied antimicrobial spectrum against both Gram-positive and Gram-negative bacteria, mycobacteria, viruses and fungi [15,16]. Nonetheless, there is currently little information on the manner in which antimicrobial agents influence pathogens, resulting in the disruption of the normal cell membrane, growth inhibition and even cell death. Specifically, the significant activity of AMPs against *M. tuberculosis* has viewed them as prototype molecules in the planning of novel anti-TB agents [17]. In this review, several natural AMPs from varying organism sources with a wide range of *in vitro* and *in vivo* cidal activity against *M. Tuberculosis* are detailed, even while their overall mechanism of action is debated.

2. Need for new anti-tuberculosis drugs and schemes

Even though the presently available treatment regimens for the drug-sensitive tuberculosis are greatly efficient subject to patients' adherence under ideal conditions, the results are not perfect when the actual life realities of tuberculosis programmatic conditions are considered [18]. The WHO's suggested treatment regimens for drug-susceptible and drug-resistant tuberculosis are saddled with several inherent challenges, rendering novel antituberculosis drug discovery a priority for clinical and public health [19–24].

The first challenge is the protracted nature of the treatment regimen for the drug-susceptible disease, which extends over a minimum period of six months [25]. Additionally, the first-line oral drugs (isoniazid, ethambutol, pyrazinamide, and rifampicin) have to be taken simultaneously in the first two months of treatment, while isoniazid and rifampicin are taken over a consecutive period of four months during the continuation stage, resulting in the challenges linked to patients' adherence to the regimens. These regimens are associated with high mortality rates and patients' non-adherence. They can lead to the development of chronic drug-resistant tuberculosis [23,26]. Therefore, a key concern for drug development has been for novel tuberculosis agents, which will reduce the treatment regimen. Strong sterilizing agents, which may reduce the treatment period to two months and below, could enhance adherence and decrease the cost of distribution and programme supervision [27]. In addition, the drugs that could decrease the total duration of the treatment and regularity of drug intake are always preferable. Studies on the life cycle of *M. tuberculosis* have illustrated that mycobacteria generates a dormancy phenotype under anaerobic conditions and nutrient deprivation [28–31]. The bacterial population of the persisters may endure for a maximum of 100 days following the commencement of anti-tuberculosis treatment, while a reviving element is needed for duplication in order to encourage a swift reproduction of the inactive bacilli. Such dormant bacteria are not susceptible to numerous anti-tuberculosis agents and can lead to an extension of anti-tuberculosis treatment. Therefore, novel drugs and regimens are required for a complete denaturing of all the persisters, regardless of the developmental stage attained by the *M. tuberculosis* [29,32–34].

Secondly, novel drugs are required to deal with the increasing global challenge of MDR-TB and XDR-TB [35]. MDR-TB, which results from the resistance of the *M. tuberculosis* to rifampicin and isoniazid, is currently widespread at a global level, with around 500,000 cases documented in 2015 [36]. XDR-TB (as a result of *M. tuberculosis* resistance to isoniazid, rifampicin and any fluoroquinolone, as well as at least one of the three injectable second-line medications: kanamycin, amikacin, or capreomycin) has been documented in many countries. Patients with MDR-TB require a combination of second-line and third-line anti-tuberculosis agents [37], which are considerably costlier, noxious and less efficient than regular treatment. The WHO's recommendations for addressing MDR-TB and XDR-TB refer to second-line drugs and a treatment period covering more than 18–24 months [23]. These directions are based on low-grade evidence, experts' viewpoint and little observational information, and do not have the rigour of evidences according to the data from randomized trials. The implementation of such directions comes with a broad array of treatment regimens, which rely on the availability of drug-susceptibility assessments, expenses, doctors' preference and the availability of drugs in developing countries. Therefore, there is a need for novel regimens for MDR-TB and XDR-TB, which are less protracted, more bearable, more efficient and have been assessed under programmatic conditions [38,39].

Thirdly, anti-tuberculosis agents may interact with antiretroviral therapy (ART), thereby presenting a great control challenge in sub-Saharan Africa, where the cases of TB are mostly driven by

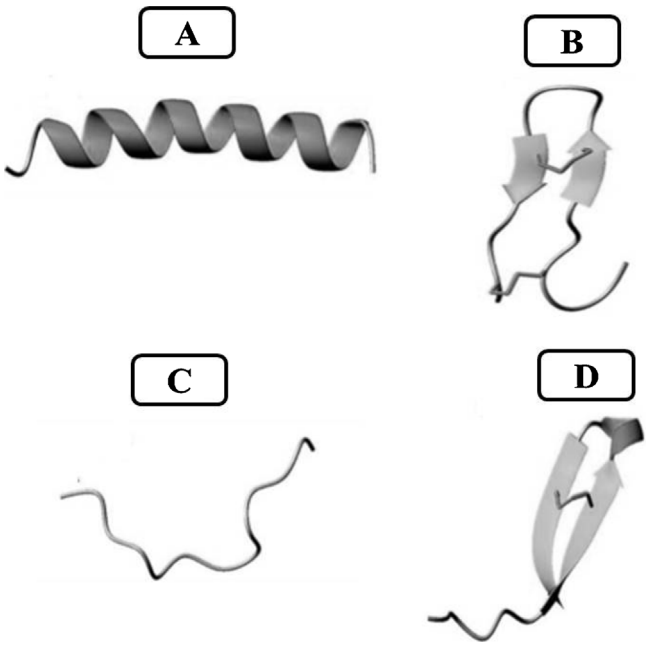

Fig. 1. Secondary structures of selected AMPs: A) α-helical, B) β-turn/sheet, C) random coil and D) mixed α/β.

the spread of HIV [40]. Fourthly, novel treatments are needed for enhanced patient therapy, especially for those suffering from the latent infection of tuberculosis prior to its transformation into an active state. The WHO estimated that around two billion people have LTBI [41], while over 100 million people with the dormant tuberculosis infection will develop active disease in their lifetime [42,43]. Even though the occurrence of tuberculosis is comparatively reduced in developed countries, the reactivation of the latent tuberculosis infection in local and migrant societies contributes to a great proportion of the TB burden [44]. The novel anti-tuberculosis drugs and the alternative intervention tactics for the enhanced treatment of latent TB infections are considered as the best approaches towards the eradication of TB. Thus, there is a need for an adequate safety profile of the novel anti-tuberculosis agents to be more potent than the available agents, capable of reducing the duration of therapy, efficient in the treatment of MDR-TB and XDR-TB and compatible with simultaneous ARTs, as well as involve no adverse interaction with the alternative tuberculosis agents in the treatment regimen. To treat latent TB infections, the agents have to competently act against the different replications and physiological states of *M. tuberculosis* [43,45].

3. Classification and physiochemical property of naturally-occurring AMPs

AMPs are generally considered as positively charged small peptides, comprising about 12 −50 amino acids [46–48]. The availability of the positively-charged amino acids, such as lysine and arginine, confers a general positive net charge to the AMPs. The AMPs have hydrophobic and hydrophilic regions, which make them amphipathic in nature, facilitating their interaction with biological membranes while sustaining their solubility in aqueous environments [49,50]. AMPs are classified into four categories in line with their secondary structure: α-helix, β-sheet, extended helix and loop (Fig. 1). The amphipathic α-helix AMPs are the most regularly and broadly researched group, comprising estimated 27% of all AMPs with renowned secondary structures [50,51]. In the α-helix AMPs, the hydrophobic amino acid residues are isolated from the hydrophilic residues and gathered on one side of the helical wheel, leading to the formation of the hydrophobic side of the helical wheel [10,48,52]. Even though the majority of the AMPs are part of the four classes stated previously, their secondary structures may alter as a reaction to varying environments. Several AMPs take on various structures when dealing with biological membranes to facilitate the separation of the hydrophobic face from the hydrophilic face [53]. For instance, cationic AMP indolicidin changes from a globular arrangement in aqueous solutions to a wedge-shaped arrangement, surrounded by two dispersed and positively charged hydrophilic regions in a membrane-mimic environment [49,52,54].

4. Structure-activity relationship of AMPs

AMPs mainly exert their actions through immediate interaction with microbial membranes. Thus, the specificity and discerning noxiousness of AMPs towards the microbes over the host are very significant. The activity of AMPs is impacted considerably by the physiochemical features and structural arrangements, encompassing the length, general charge (Q), hydrophobicity (H), charge angle (θ), conformation (χ), amphipathicity and solubility [55,56].

The activity of an AMP involves a function of its length because the formation of amphipathic structures with hydrophobic and hydrophilic faces on the opposite sides of a peptide molecule requires at least seven to eight amino acids. In the barrel-stave model, at least 22 amino acids are required for α-helical AMPs to permeate the lipid bilayer of bacteria, while β-sheet AMPs require eight amino acids [57]. In addition to the effect of AMP length on its mode of action and 3D structure, it can also affect the level of its cytotoxicity. For instance, a 300% lower toxicity was reported when rat erythrocytes and human erythrocytes were exposed to a shortened melittin with 15 residues at its C-terminal [58] and a shorter derivative of HP (2–20) [59], respectively, compared to their unaltered forms. Therefore, it is necessary to consider the length of an AMP when designing new synthetic peptides with low toxicity. Moreover, the efficacy of ATRA-1A isomers has been investigated against selected Gram-negative and Gram-positive bacteria at low and high salt conditions. The EC50 values of L-ATRA-1A and D-ATRA-1A at low salt values against *E. coli* were 4.3 μg/mL and 1.4 μg/mL, respectively, but ∼9.7 μg/mL and ∼7.1 μg/mL respectively, at high salt values. Against *B. cereus* (Gram-positive bacterium), the EC50 values of L-ATRA-1A and D-ATRA-1A, at low salt conditions were 73 μg/mL and 2.3 μg/mL, respectively, while being completely ineffective against *B. cereus* at higher salt values. The shorter D-ATRA-1A isomer, therefore, showed more potency than the L-isomer [60].

The electrostatic interaction among cationic AMPs, as well as the negatively charged elements of bacterial membranes, are responsible for the initiation of antimicrobial activity. Thus, the net charge of AMPs contains one of the precursors of AMP activity. Usually, AMPs have abundant lysine or arginine, with a general net charge of between +2 to +11 [10,56]. Several studies have shown the association between peptide charges with their antimicrobial activity [61–65]. An increase in the activity of magainin II amide analogues has been portrayed as improving the net charge from +3 to +5 [66,67]. Corresponding to the result involving magainin II, studies with human defensin have additionally portrayed a direct association between the charge density and the antimicrobial activity [68,69]. A change in the net charge of an AMP can alter the antimicrobial and haemolytic activities of the AMP towards achieving a selective effect on microbes with minimal toxicity for the host cells. A higher haemolytic activity was achieved by increasing the positive net charge of V13 K from +8 to +9, while decreasing the net charge to lower than +4 neutralized its effect against *P. aeruginosa* [70].

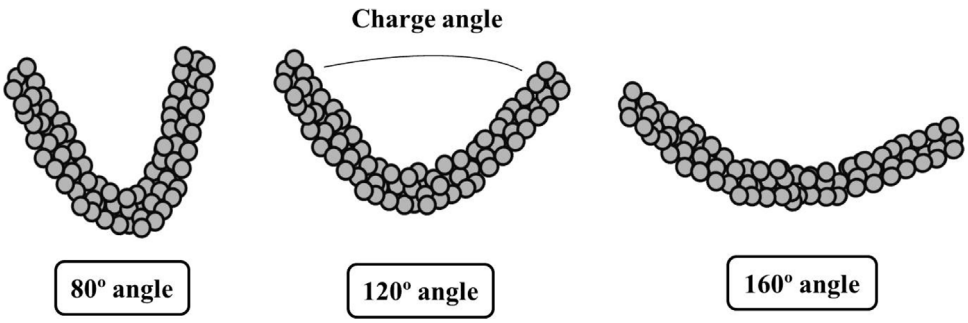

Fig. 2. Diagram illustrating the charge angle of α-helical peptides.

The overall hydrophobicity of the AMPs involves a further study on their essential structures. Every amino acid has its own relative hydrophobicity index. The general hydrophobicity of a peptide may be computed through the inclusion of the hydrophobicity index of the amino acids contained therein [71,72]. Hydrophobicity is regarded as autonomous from the alternative parameters [10,73,74]. The studies conducted on the C3a- derived peptide CNY21 showed that the adsorption of AMPs through lipid bilayers involved a driven compulsion of the membrane disturbance impact of the AMPs, while the hydrophobicity has been found to determine the degree of adsorption [75]. Generally, an increase in the hydrophobicity of AMPs will result in increased antimicrobial activity [76]. Magainin, for instance, is the only AMP that is effective against Gram-negative bacteria, although some synthetic analogues with higher hydrophobicity can be effectives on eukaryotic cells and some Gram-positive bacteria as well [62]. Nonetheless, a high hydrophobicity may additionally bring about peptide aggregation or precipitation on the exterior of the membrane, thereby restricting the active concentration of the AMPs required for membrane interaction and ultimately decreasing their antimicrobial activity [77]. The threshold hydrophobicity of AMPs has been noted as being responsible for selective bacterial membrane inclusion. A higher hydrophobicity, in contrast to the threshold hydrophobicity, may result in reduced antimicrobial efficacy and increased noxiousness in mammalian cells [65,68,78].

In the case of the helical AMPs, the hydrophobic and hydrophilic residues separate and converge on different sides of the helical wheel [79]. The charge angle (Fig. 2), which is considered to be an essential measurement of the relative portion of the polar/hydrophilic to non-polar/hydrophobic sides in helical AMPs, comprises the angle subtended by the positively charged residues [80]. Several studies have reported that the charge angle affects the membrane permeation and pore formation capability of AMPs, as smaller charge angles seem to have a greater permeability through biological membranes [62,80,81].

AMPs may assume different secondary structures such as α-helix, β-sheet, loop and extended helix [47]. The conformation assumed by AMPs in the presence of other biological membranes is an extended key towards the determination of their efficacy and selective noxiousness [82,83]. Proline residue is available in the central region of numerous naturally occurring AMPs, such as piscidin, gauguin and buforin II (BF2) [84,85]. The studies on BF2 have shown that the availability of a proline residue in the sequence is essential for its function, impact on membrane transposition, permeabilization and possible intracellular targeting. A reduction in activity was noted from these peptides following proline mutation [86–88]. Several studies have signified that proline residues may destroy the local helical structure, thus, raising the conformation amenability of AMPs. The availability of proline may additionally destabilize the self-association of AMPs, enabling their transposition over the interior membrane. Furthermore, the presence of proline residues offers some degree of selectivity to the AMPs [82,85]. The location of the proline is additionally essential. It was noted that transporting proline in the direction of either the N- or the C- terminus of BF2 reduced the transposition of the peptide over the membrane [89]. The integration of a single proline residue at the centre of the hydrophobic countenance of AMPs conveys flexibility and facilitates the transposition and permeabilization of AMPs over the bacterial membranes. The activity of AMPs is not established by a single element, but a combination of a number of structural parameters.

Another essential property of AMPs towards ensuring their interaction with microbial membranes is their amphipathicity, which has been shown by Fernandes-Vidal et al. [90] to be more important than hydrophobicity for the binding of AMPs to microbial membranes. A greater priority should be accorded to the amphipathic structure of AMPs when designing synthetic AMPs for specific target cells, since the amphipathicity of AMPs is required for a strong partition into the membrane interface [90].

AMPs need to be soluble in aqueous environments in order to enter lipid membranes. AMP molecules lose their ability to interact with the cell membrane when they aggregate. For instance, there is a high tendency of a hybrid synthetic AMP, made up of cecropin and melittin, to form dimers. Deamination can be prevented on the nonpolar surface of this hybrid AMP by substituting a Lys residue, leading to a reduced haemolytic activity. This AMP is more effective in its incorporation into microbial membranes by losing its dimerization capability [91]. The importance of solubility and structural optimization has, therefore, been demonstrated by this example.

5. Mechanisms of action of AMPs

Human cathelicidins and defensins can kill a broad range of Gram-positive and Gram-negative bacteria [92–97]. The mechanisms, which are mostly acknowledged, consist of the barrel-stave model, the toroidal model and the carpet model for killing organisms. In the barrel-stave model, the peptides arrange themselves in order to bind to the cell membranes, which results in the amassment and transformation of the peptide into a bilayer (Fig. 3A). In this manner, the hydrophobic peptides are arranged in line with the lipid centre, while the hydrophilic peptides create an access pore in the internal section of the membrane. The carpet model involves a disruption of the membrane through peptide binding to the external surface of the cell membrane, thereby creating a prolonged layer or carpet. In the toroidal model, the attached peptides begin to collect and compel the lipid monolayer to constantly bend through the pores [98,99]. In this manner, the core is lined not only by the inserted peptides, but also by the lipid head groups [46,65,100]. Several authors have disclosed that the AMPs have several modes of action. Carrol et al. [101] stated that the antibiotics nisin and lacticin 3147 have various mechanisms of action for binding to peptidoglycan for the subsequent prohibition of the biosynthesis of

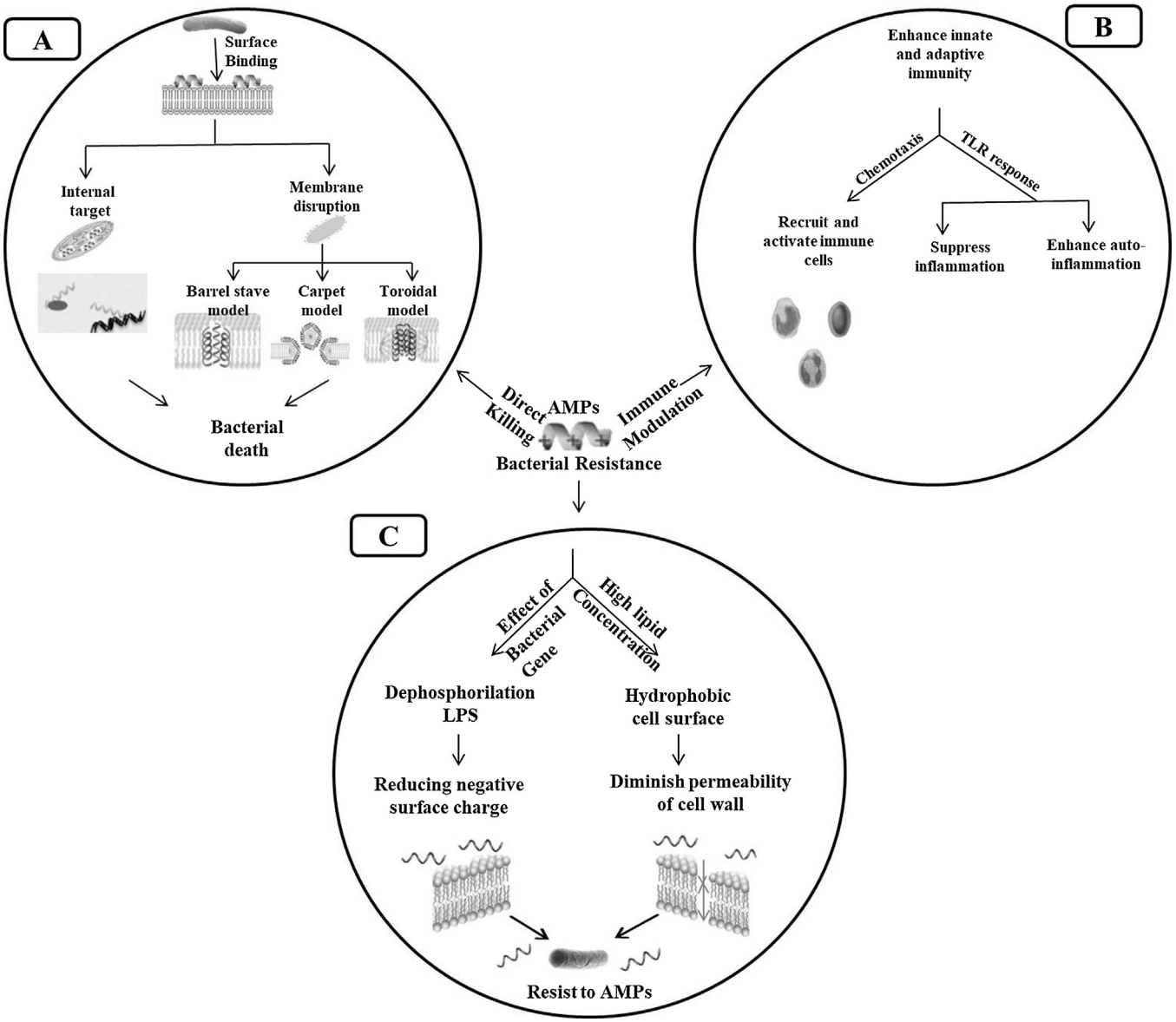

Fig. 3. Mode of action used by AMPs to induce the killing of *M. tuberculosis* and the mechanisms of bacterial resistance against AMPs.

peptidoglycan and creation of pores in the membrane of the target cells [101]. It has additionally been demonstrated that uridyl peptide antibiotics may inhibit the bacterial translocase MraY included in the bacterial cell membrane, thus showing activity against *M. tuberculosis*.

Each of the named models portrayed membrane disruption as the mechanism of bacterial killing. Nonetheless, AMPs may target key intracellular processes, which result in bacterial death without disturbing the membrane. Additionally, AMPs have been observed to cross cell membranes and inhibit crucial cellular processes without causing considerable membrane disturbance [46,102,103]. Therefore, the human defensin analogues were illustrated to travel across the interior and exterior *E. coli* membranes without resulting in cell lysis, implying that the noted bactericidal action could depend on cytoplasmic interactions [104]. The bovine cathelicidin indolicidin, which includes a broad spectrum of bactericidal action, has been illustrated to not only lyse bacterial cells but also block DNA synthesis [105], as well as shown to interact with duplex DNA *in vitro* [106]. The buforin AMP analogues were additionally shown to exert bactericidal action against *E. coli* by binding to the RNA and DNA after breaching the cell membrane [107].

In addition to the indirect effects of killing intruding microbes, there is a possibility that AMPs disturb regular cellular functions, such as gene transcription, apoptosis and cytokine production, to improve the innate and adaptive immunity of the host. This is based on their chemokine-like and immunomodulatory features of AMPs, including the chemotaxis of leukocytes [46,108] (Fig. 3B).

It has been suggested that bacteria decrease their susceptibility to AMPs through a preventive peptide attachment to the exterior membrane [109]. Recently, Cullen et al. [110] stated that the steadiness of microbes could be maintained through a single gene, which controls the dephosphorylation of LPS, thus decreasing the net negative surface charge. This confers the resistance to AMPs on bacteria through a reduction in their interactions with the bacterial cell surface [110]. The unique *M. tuberculosis* cell wall, which functions primarily to obstruct the permeability of anti-tuberculosis agents, contributes to their resistance and pathogenicity [111]. The peculiar mycobacterial cell wall contains a high lipid concentration, which confers hydrophobicity onto the surface of the cell. The

Table 1
Summary of publications showing the activity of various AMPs with conventional antibiotics.

AMPs with Antibiotics	Microorganisms	AMPs (Minimum Inhibitory Concentration/ Fractional Inhibitory Concentration)	References
DP7 + gentamicin, vancomycin, azithromycin, and amoxicillin	multidrug-resistant bacterial strains *S. aureus, P. aeruginosa, A. baumannii,* and *E. coli*	≤32 mg/L	[123]
Polymyxin B + tigecycline	*A. baumannii*	100 mg/200 mg	[124]
Plectasin NZ2114 + teicoplanin, moenomycin, and dalbavancin	*E. faecalis*	256 mg/L	[125]
Colistin + fusidic acid	multidrug-resistant *A. baumannii*	512 mg/litter	[126]
Oligo-acyl-lysyls (OAKs)+ rifampin	*K. pneumoniae*	>50 mg/mL	[127]
Bacillomycin D + amphotericin B	*Candida albicans*	12.5 Lg ml^{-1}	[128]
KR-12-a5+ chloramphenicol, ciprofloxacin, and oxacillin	multi-drug-resistant *P. aeruginosa* (MDRPA)	From 0.3125–0.5	[129]
Melittin + tetracycline	*S. aureus* *S. epidermidis* *E. coli*	4.17 mg/mL 3.75 mg/mL 5.00 mg/mL	[130]
Nisin Z + methicillin	*S. aureus* *S. epidermidis* *E. coli*	10.00 mg/mL 9.17 mg/mL 16.67 mg/mL	[130]
D-IK8+ vancomycin	vancomycin resistant *S. aureus*	0.75	[131]

major constituents of the outer layer, which plays an important role in the lipid-rich cell wall structure, are mycolic acid and long-chain hydrocarbons [112,113]. This tightly packed array with a considerably reduced fluidity causes the amassment of the cells and a subsequent decrease in the permeability of the cell wall [114,115] (Fig. 3C).

6. The action of different microbial agents is augmented by AMPs

With conventional antibiotics, synergistic effects are noted and suggested to be associated with the strong membrane-peptide interactions, which increase the permeability of the bacterial surface and enhance the uptake of intracellular drugs [116]. The concentration of the involved antimicrobial agents could be reduced, thereby decreasing the cytotoxicity of the agent and the onset of resistance. The IDR-1018, DJK5 and DJK6 and the synthetic peptides have shown a synergistic action against several Gram-negative pathogens. These combinations are made up of one or several of the conventional antibiotics, such as tobramycin, ciprofloxacin, imipenem and ceftazidime [117,118], with a decrease in their efficient concentrations by up to 64 folds. The IDR-1018 further showed a synergistic action with chlorhexidine, an antiseptic agent, against multispecies of biofilm. Similarly, the activity of carbapenem and imipenem against plasma-controlled carbapenemase-generating *Klebsiella pneumoniae* was enhanced by DJK-6, outlining the manner in which peptides may be employed in the place of antibiotics [119,120]. In addition to their synergy with antimicrobial agents, peptides may additionally be used to widen the antibiotic range. The efficacy of vancomycin has improved against MRSA in rat models through a combination with the cathelicidin peptide BMP-28 [121].

Recently, Mishra *et al.* [122] conjugated CRAMP (murine cathelicidin) with vancomycin and reported that the resultant combination acted antagonistically against the studied Gram-positive and Gram-negative species. Conversely, the activity of the conjugate was greatly enhanced by an equimolar combination of vancomycin and CRAMP, signifying the advantage of the covalent linkage. The authors theorized that CRAMP assisted in the translocation of vancomycin into the periplasm of the Gram-negative bacteria, showing that peptides may assist in the repurposing and broadening of the antibiotics range [122]. Table 1 summarizes the most recent studies of synergistic combinations between AMPs and antibiotics

7. The putative intracellular targets of AMPs in mycobacteria

M. avium and *M. intracellulare* are sensitive to human a-defensin-1 (hNP-1), hNP-2 and hN*P*-3. The agent has also led to the killing and clumping of the organisms [132]. Killing has been shown by Ogata et al. [132] to be independent of the strains' colony morphology. Ten different strains were sensitive to hNP-1 with a killing rate ranging from 34.2% to 87.2% at an hNP-1 concentration of 50 μg/mL. They further showed that the bacteria have a limited growth rate at pH 2 and 4; but, as the pH increases, the rate of growth increased as well [132]. There was no evidence of a strong reliance on the activity on the tested salt concentrations (calcium and magnesium were tested at concentrations of 0.5 and 1 mM, while NaCl was tested between 25 and 100 mM). Mice exposed to experimental tuberculosis (1.5×10^4 CFU of Mtb H37Rv hNP-1) also showed sensitivity to hNP-1 [133]. The hNP-1 was injected into the mice subcutaneously, which showed evidence of *M. tuberculosis* in the lung, liver and spleen of the mice. It has been reported that, under scanning electron microscopy, about 70–100 nm "warts" could be seen in hNP-1-treated mycobacteria within 20 min of exposure [134]. The authors referred to these "warts" as lesions. Lesions usually describe membrane-spanning pores formed from different proteins. However, the images on the SEM cannot authoritatively confirm the formation of lesions on the mycobacterial cell envelope. Other intracellular targets were also discussed outside the mycobacteria cell envelope and membranes. An induction of DNA injuries from hNP exposure was also found to be possible [135]. It has been documented that hNP-1 appears to bind to the *M. tuberculosis* plasma membrane/cell wall and, as a secondary target, to the bacterial DNA [136]. In the DNA biosynthesis, a maximum cytosolic macromolecules inhibition of 75% was observed [136].

Sunshine and UV light are known to play a crucial part in the management of *M. tuberculosis* cases. Vitamin D in the active form of $1,25(OH)_2D3$ has been shown by Rook and Crowle [137,138] to be an important element for human monocyte and macrophage antimicrobial activity against *M. tuberculosis* [137,138]. It has also been shown that a TLR-2/-1 MTB-derived 19-kDa lipopeptide ligand can stimulate a vitamin D-dependent pathway, which could induce human cathelicidins [139] and killing activity against intracellular *M. tuberculosis* [140]. The authors also demonstrated that, through the use of a single siRNA against cathelicidins, $1,25(OH)_2D3$-induced antimicrobial activity can be inhibited and

alternatively lead to an increased intracellular growth of *M. tuberculosis* [141]. The addition of vitamin D to THP-1 cell cultures can also induce human cathelicidin production [142]. The simultaneous addition of vitamin D and *M. marinum* NJB0419 has been shown by Sato et al. [142] to be important for the accelerated production of hCAP18. The proliferation of *M. marinum* in THP-1 cells can be decreased by the induction of CAP18 production or through an externally sourced LL-37. The antimicrobial effects of $1,25(OH)_2D3$ and external LL-37 against *M. marinum* infection in THP-1 cells have been abrogated by autophagolysosome inhibitors [142]. The phagocytic action against intracellular bacilli is mediated by NOX2 through the production of reactive oxygen species (ROS). NOX2 has been shown by Yuk et al. [143] to be essential for 1,25D3-mediated antimycobacterial activity via cathelicidin expression [143]. Innate immunity and autophagy mediated by D3 have been shown by Yuk et al. [143], via a regulatory function of LL-37, to be important in the degradation of damaged cytosolic components and misfolded proteins [143]. They further showed that the colocalization of mycobacterial phagosomes with autophagosomes can be induced by 1,25-D3 in an LL-37-dependent way. It was thus concluded that antimycobacterial activity depend on being mediated by 1,25-D3 and required LL-37 and autophagy [143].

Rivas-Santiago et al. [144] morphologically studied the effect of LL-37, CRAMP and related synthetic peptides on the morphology of *M. tuberculosis* using electron microscopy [144]. All peptides were found to induce the destruction or modification of cell walls and the condensation of cytoplasmic components. There was a homogenous increase in the electron-lucent cell wall, which is surrounded by a thin electron-dense rim. It has been concluded that the cell wall and the membrane are the prime cathelicidin-based peptides targets, and that osmotic activity, cell wall synthesis disruption and maybe DNA binding can take place [144].

PR-39 is an AMP, rich in proline and arginine, and isolated from the intestine of pigs. It was found to be active against the drug-susceptible and MDR-TB clinical isolates, while, at a concentration of 50 μg/mL, to possibly inhibit the growth of *M. tuberculosis* H37Rv by 80% [107]. The E1380/94 and P34/95, which are MDR-TB strains, were less sensitive to PR-39; a growth inhibition of 39 and 49% was observed at a peptide concentration of 50 μg/mL, respectively. Against *E. coli,* the activity of PR-39 was not dependent on the permeation of the membrane, but on DNA inhibition and synthesis of proteins [145].

Ubiquitin, which is released into the circulation upon stimulation of chromaffin cells, is stored in the secretory chromaffin granules of the cells [146]. The lysosomal killing of *M. tuberculosis*, mediated by ubiquitin-derived peptides, which were generated by hydrolysis, was reported by Alonso et al. [147]. The induction of autophagy in infected macrophages can increase the delivery of ubiquitin conjugates to the lysosome. The full-length ubiquitin has no antibacterial activity. However, bactericidal activity against *M. tuberculosis* has been reported in ubiquitin-derived peptides from a cathepsin digest of purified ubiquitin or a synthesized peptide Ub2 (STLHLVLRLRGG) [148]. The bactericidal activity of the ubiquitin is dependent on the interaction between the Ub2- ubiquitin-derived peptide and the mycobacterial cell envelope. Four *M. smegmatis* mutants were found by Purdy et al. [149] to be resistant to the bactericidal action of Ub2 and other AMPs. Their susceptibility is independent of the MspA channel activity, but relies on the reduced permeability of the cell wall when compared to the wild-type bacteria [149]. Ub2 has a β-sheet structure in SDS micelles and POPC: POPG (1:1) liposomes [150]. It enters lipid membranes and permeates liposomes, as demonstrated by a carboxyfluorescein leakage assay. The authors mimic the inner membrane of mycobacteria using this lipid composition. The transmembrane potentials in the fast (*M. smegmatis*) and slow (*M. bovis* BCG) growing bacteria can be eliminated by Ub2. The Ub-peptides are localized in the cytoplasm and membrane of mycobacteria, although intracellular targets remain possibility [150].

Sharma et al. [151] synthesized and used AMPs (SAMPs; MW = 45–50 kDa) consisting of biodegradable polyamide backbone and different amounts of basic dimethylamine and imidazole pendants. Probably due to the inhibition of cellular uptake by the outer layer of the outer membrane (mainly lipopolysaccharides), the SAMPs have no bactericidal activity against *E. coli*. They are potent against the rapid growing *M. smegmatis* with a growth inhibition rate of 85%, which is achieved for the most active peptide at a concentration of 15 μg/mL. The binding of SAMPs to bacterial DNA has been proposed by Sharma et al. [151] as the mode of action of the SAMPs [151]. Under a fluorescent-labelled SAMP study, peptides have been shown to penetrate the mycobacterial cell wall, as well as epithelial cell (A549) membranes, but never the *E. coli* cell envelope. There was no cytotoxic effect as a result of the permeation of the SAMPs into A549 cells. This neither caused cell membrane permeation nor induced morphological changes. However, the authors speculated that the binding of the SAMPs to the DNA hindered the DNA replication and cell death as a consequence [151].

8. Activity of AMPs against *M. tuberculosis in vitro* and *in vivo*

8.1. α-helical peptides

Some AMPs have been found to have an α-helical structure. The selectivity and potency of α-helical AMPs greatly rely on their physiochemical properties, such as their charge angle, overall hydrophobicity, net charge and amphipathic nature [152]. Furthermore, the use of the D-conformation peptides has been widely favoured as it has been demonstrated that the L- to D-amino acid substitution among AMPs confers resistance to the activity of proteolytic enzymes and, thus, enhances the biostability of the AMPs, without disturbing their antimicrobial activity [153,154]. The peptide D-V13 K (D5) consists of a 26-residue amphipathic peptide, made up of all D-amino acid residues, assumes an α-helical conformation located in a hydrophobic environment, while comprising hydrophilic and positively charged valine and lysine at position 16 as a "specificity determinant". It has been described as the most potent analogue against *M. tuberculosis*, with an MIC of 15.6 μM (49 μg/mL) against the MDR strain, and 11.2 μM (35.2 μg/mL) against the H37Rv strain. The peptide D1, with a positively charged lysine residue at the core of the non-polar face, showed similar activity in relation to D5 against the MDR strain (57 μg/mL): a nine-fold enhancement in the haemolytic activity, with a therapeutic index that was 7.4-fold better in contrast to D5 [152]. Furthermore, D-LAK120-A and D-LAK120-HP13 showed the greatest potency, with no detection of the mycobacteria in the wells at a concentration of 50 μM and above. In comparison, D-LAK120-H and D-LAK120-AP13 displayed less activity due to the great number of available bacteria in the wells, even at a concentration of 100 μM. In the case of the MDR strain, although none of the D-AMPs was capable of eradicating the growth of the bacteria, even at high concentrations, D-LAK120 and D-LAK120-HP13 were the most potent D-AMPs against this strain, followed by D-LAK120-A and D-LAK120-H.

Regarding the XDR strain, D-LAK120 and D-LAK120-A were the most efficient peptides. Once again, none of the D-AMPs was able to inhibit the growth of the XDR strain. Overall, D-LAK120, D-LAK 120-A and D-LAK120-HP13 were considered as the most potent D-AMPs against *M. tuberculosis* strains *in vitro* [155]. A series of synthetic α-helical peptides, altered with hydrophobic amino acids, has been assessed for their antimycobacterial activity. The peptide (LLKK)2

was altered, with two methionine residues (M(LLKK)2 M) presenting the most potent peptide against susceptible *M. tuberculosis* and MDR-TB with an MIC of 125 mg L^{-1} and 62.5 mg L^{-1}, respectively [156]. Even though the exert mode of action of AMPs is vague, and may vary according to the AMP, it is apparent that AMPs positively charged residues are drawn to the negatively charged surface of the bacterial membrane.

The non-polar region of the AMP has been integrated into the lipid bilayer and the peptide amassment into the membrane, causing enhanced permeability, loss of barrier function, leakage of cytoplasmic components and cell death [152]. Additionally, the D-AMPs showed the capability to cause heavily clustered bacteria in a culture wall to appear more sparingly. This special attribute of the D-AMPs could be clarified through their amphipathic properties. Even though the general hydrophobicity of various D-AMPs differs, each of them is capable of generating a 'detergent-like effect' through decreasing the hydrophobic interaction between the bacterial cell walls and hindering cell clumping [155].

It has been established that a class of peptides from various Gram-positive bacteria (Bacteriocins (Bcns)) exert activity against a broad range of bacteria. Bcns made from lactic acid bacteria are classified into three categories, with the mode of action of those in classes I and IIa having undergone comprehensive scrutiny [157,158]. The bacteriocins Bcn1-Bcn5 have shown an enhanced anti-mycobacterial activity against *M. tuberculosis* with an MIC of 0.01–1 μg/mL [159]. The action of Bcn1-Bcn5 against *M. tuberculosis* is based on the creation of pores in the cell membranes, leading to cell death [157,158].

Nisin A, which has been acknowledged by the Food and Drug Administration (FDA) and incorporated into a wide range of commercial products, is the prototype lantibiotic. Like other lantibiotics, the synthesis of mature nisin peptides involves a considerable post-translational alteration, causing the creation of the unusual amino acids lanthionine and β-methyllanthionine, in addition to dehydrated amino acids. Regarding the clarification of the significance of particular domains and residues contained in the peptides, lantibiotics bio-engineering has been especially useful. Specifically, the N-terminal region, which is responsible for the binding of lipid II in the cell wall of targeted cells, was clarified, as was the terminal region, which is inserted into the membrane of the cell to form pores. The most significant aspect is the area that focused on the central hinge region, which permits the previously stated domains to travel relative to each other. Conversely, the production of lantibiotics with improved structures has not been regularly stated, although several breakthroughs have been observed, such as the production of nisin variants with enhanced solubility at neutral pH or enhanced antimicrobial action against non-pathogenic strains. The function of nisin A and the new bio-engineered hinge derivatives, nisin V, nisin T and nisin S, have been assessed. Each of the variants signified an additional antimicrobial activity contrary to nisin A. Nisin S presented as the most potent variant against *M. tuberculosis, M. kansasii* and *M. avium*, inhibiting their growth by a maximum of 29% when compared to nisin A [160].

Lactoferrin, which is available in mucosal secretions and neutrophilic granules, is an iron-binding glycoprotein. Additionally, it is considered as a fundamental element of the innate immunity and has the capacity to alter host reactions in *M. tuberculosis* infection [161]. Mice administered with lactoferrin displayed a 1 log10 decrease in bacterial load after three weeks of oral administration. The positive effects were apparent, even when the mice received the treatment one week after infection, signifying that lactoferrin is a promising new agent for the treatment of TB [162]. It was demonstrated by quantitative immunohistochemistry using multispectral imaging that lung inflammation was greatly decreased in the two groups of mice treated with lactoferrin. A reduced foamy macrophage, enhanced total lymphocytes and an increased number of CD4þ and CD8þ cells were also reported. An ELISpot analysis showed that mice treated with lactoferrin had increased CD4 þ IFN-gþ and IL-17 generating cells in their lungs; these cells have conferred protection during *M. tuberculosis* infection. Lactoferrin on its own did not alter the growth of *M. tuberculosis* in the macrophages or broth culture, but augmented the IFN-g mediated killing of the *M. tuberculosis* through the macrophages in a nitric oxide-mediated pathway. Therefore, lactoferrin could become a new therapeutic agent for the treatment of TB and be beneficial for the decrease in immune-mediated tissue damage in infectious diseases [162]. Mice infected with H37Rv and MDR-TB, given 32 μg/mouse of LL37 three times a week for 28 days, showed approximately 53% and 45% of *M. tuberculosis* and MDR-TB killing, respectively. These findings signify that AMPs could be a new treatment agent against TB [144].

Additionally, the *in vivo* killing of *M. tuberculosis* prompted by LLKKK18 (AMP)-loaded HA nanoparticles with a 1.2-log decrease at 100 μM of C57BL/six mice, underwent infection with *M. tuberculosis* through the pulmonary route. After three months, five doses of the treatment were administered intratracheally on a daily basis [89].

A major class of mammalian AMPs, which is known to be an important aspect of the innate immune system that provides a non-specific, rapid response to foreign pathogens, comprises cathelicidins. Although cathelicidins have been demonstrated to have a direct *in vitro* antimycobacterial effect [163], information on the cathelicidin-dependent immunoregulation of other immune responses of the innate and adaptive systems is still limited; they are simply regarded as serving as chemotactic factors for CD4T cells, neutrophils and monocytes [164,165]. Cathelicidins are hereby strongly suggested as important multifunctional host immune regulators, which have direct antimycobacterial activity and an immunoregulatory influence on adaptive immune responses against *M. tuberculosis* via the modulation of pro-inflammatory cytokine responses, apoptosis and calcium influx. This protective role of cathelicidins during TB infection, which depends on the *M. tuberculosis*-mediated cAMP burst in macrophages, provides insight into the pathogenesis of TB and suggests a further investigation into cathelicidin-mediated immune regulation [166].

To improve the helical structure and antibacterial activity of LL37, LLAP (which is a 15-amino acid LL37-analogous peptide with the amino acid sequence–GRKSAKKIGKRAKRI) was designed and compared to the native sequence [167]. The designed LLAP showed a high basic amino acid (arginine and lysine) content, with an improved positive charge and pore forming characteristics due to the presence of lysine in the C-terminal [167]. The amino acid sequence of both the native and the LLAP peptides was predicted to adopt an alpha helix structure due to the projection of helical wheels and secondary structures [167]. The IC_{50} of LLAP and the native peptides were 11226 μg/mL and 7727 μg/mL, respectively. This was about 13.8 and 9.5-fold less, respectively, than the kanamycin used as a positive control [167]. LLAP had a lower haemolytic activity of less than 1.1%, while the same level of cytotoxic activity was observed for the two peptides [167]. There could be flexibility in configuring the amphipathic regions by the two peptides, despite not showing a full helical structure. As such it is possible that the long-side-chain positively charged amino acids, similar to lysine, may show positive charge repulsion, leading to helical structure distortion [167]. Based on the established AMPs' action mechanisms, the peptide-membrane interaction can be enabled by the high positive charge of peptides [168]. Several substitutive amino acids domiciled in LLAP, such as Gly, Ala, Lys and Arg, may increase their positivity and amphipathicity, as indicated by the helical wheel. The Phe of the native amino acid sequence

replaced the N-terminal of LLAP in this study, thereby stimulating the analogous peptide-mycobacterial cell membrane interaction as previously observed [169,170]. The interaction between the LL-37 derived peptides and the phosphatidylglycerols of bacterial cell membranes have been reported to be facilitated by the Phe in positions 1 and 12 of the native peptide [170]. Approximately 50% inhibition of the basal ATPase activity of the mycobacterial plasma membrane was attributed to the activity of the LLAP peptide, which, in turn, could be associated with impaired cell viability [167]. LLAP could be suggested, from the outcome of the study, as potential antimycobacterial compounds against cell membrane targeting ATPases [167]. A comparable IC50 was established between the peptides, although the haemolytic activity of LLAP was lower than that of the LL37 native amino acid sequence. This was significant in the inhibition of the basal ATPase activity of the mycobacterial plasma membrane [167]. Biological processes, such as the transportation of ions and other substances across the plasma membrane, were affected by this enzyme inhibition [171]. The leaching of essential substances for mycobacterial survival can be promoted through the AMPs' ability to create pores across the plasma membrane of the cells. The possibility of AMPs to anchor on the cell surface or other potentially translocated substances, which may cause an imbalance in the delicate cell-ion homeostasis, cannot be overruled. However, further crystallographic and other analytical experiments are needed for the confirmation of these possibilities [167]. Meanwhile, the MIC of LLAP (600 μg/mL) was high compared to 8–16, 0.025, 0.5, 2 and 25 μg/mL of rifampicin, isoniazid, ethambutol, capreomycin and cyclosporine, respectively, and 2 μg/mL for kanamycin (control). Therefore, there is still a need to improve the activity of the peptides [172,173].

Various physicochemical properties such as peptide hydrophobicity, charge, secondary structure and amphiphilicity, modulate the interaction of peptides with bacterial membranes [174]. In this study, synthetic peptides were designed with minimal sequence modification (to maintain their charges and amphiphilicity) in order to elucidate the influence of hydrophobicity on their antimicrobial activity. The peptides M(LLKK)2 M, C(LLKK)2C, P(LLKK)2P, I(LLKK)2I and W(LLKK)2W were obtained from the five selected (methionine, cysteine, proline, isoleucine and tryptophan) amino acids, respectively [175]. To improve the antimicrobial activity of the peptides by conferring with an extra net positive charge, the C-terminal of the six analogues was amidated [176,177]. From the six analogues, LK and PP presented the lowest α-helical contents of about 9%, while the other four peptides had an α −helicity that was three to four folds higher [175]. From the studies, it is suggested that an increase in the peptide hydrophobicity can increase the α-helical structure of the peptide. This is consistent with previous studies that reported increasing hydrophobicity in line with an increase in α-helical content [78,178]. The hydrophobicity of PP did not conform to this trend when compared to that of LK, which did not improve in propensity for α-helical folding. This means that there are other factors than peptide hydrophobicity that can modulate the helical propensity of proline-substituted analogues [175]. The steric hindrance induced by the bulky pyrrolidine ring on proline residues may be a possibility, giving rise to the distortion of the conformation in the preceding helical turn, which in turn hinders stable α-helix formation [179,180]. The presence of proline in the centre of the helices or near the C-terminal is another possibility, which can lead to the breakage of the adjacent hydrogen bonds and premature helix termination [181,182]. This could mitigate the effect of increasing hydrophobicity on the α-helicity of the peptide, giving rise to the formation of identical α-helical contents as in the case of LK and PP. Against *M. smegmatis*, the peptide analogues showed varying levels of potency with MICs ranging from 62.5 to 250 μg mL-1 [175]. Three analogues with improved antimicrobial activity were produced by the hydrophobic modification of LK, while the other two analogues were less effective against bacteria. The effectiveness of LK was enhanced by the incorporation of tryptophan, methionine and isoleucine residues into the N- and C- ends of the peptide, as demonstrated in the MIC decrease from 125 to 62.5 μg mL-1 for WW, MM and II [175]. That said, the hydrophobicity enhancement of α-helical peptides was found to improve its antimicrobial activity. High peptide hydrophobicity is associated with stronger peptide self-association, which results from the formation of dimers/oligomers formation [78,183]. Since peptide dimers/oligomers do not readily permeate through the bacterial capsule and cell wall in order to reach the plasma membrane, there is always a weaker degree of antimicrobial activity among peptide molecules experiencing high dimers/oligomers formation. This is why most high hydrophobic peptides, such as WW, do not present an improved MIC against *M. smegmatis* when compared to less hydrophobic analogues, such as MM and II. Despite increased hydrophobicity and α-helicity, a reduced antimycobacterial activity was observed with CC, compared to LK. When compared to WW, MM and II, PP had reduced antimicrobial activity, which was likely due to its reduced propensity towards helical formation [175]. The antimicrobial efficacy and activity spectrum of α-helical peptides have been shown to be reduced when there is a decrease in the α-helicity because of the introduction of proline residues into the helix [184], which suggests that peptide helicity is another vital factor that should be considered when formulating more potent peptides. A careful substitution of proline residues into peptide sequences has recently been shown to reduce the cytotoxicity effect and improve antibacterial selectivity [82]. LK, PP and II were found to be the least cytotoxic analogues with cell viabilities of more than 85% at higher concentrations of up to 250 μg mL-1 [175]. WW was the most hydrophobic and most cytotoxic analogue, with the greatest cell viability reduction from 92.3 to 13.2% and a concentration increase from 3.95 to 250 μg mL-1. About 50% of WW-treated cells remained viable at the MIC concentration of 62.5 μg mL-1, while MM and II with similar MICs had higher cell viabilities of 77.9% and 98.5%, respectively. As observed among haemolytic activities, peptide hydrophobicity was found to be more significant in relation to peptide cytotoxicity than the α-helical character [175]. This result is consistent with previous findings, which reported that an increase in the hydrophobicity of α-helical peptides can induce greater peptide cytotoxicity against RAW 264.7 cells [185]. The rate and extent of peptide-mediated membrane permeabilization have been demonstrated under flow cytometry to increase along with hydrophobicity and α-helicity enhancement [175]. This corroborates the hypothesis that peptides and rifampicin synergism are likely mediated via peptide-induced pore formation. The membrane lytic mechanism of the peptides is supported by the rapid concentration-dependent membrane depolarization leakage of intracellular ATP and the calcein release from PE/PG LUVs [175]. These findings collectively suggest that the hydrophobicity and α-helicity of peptides have a significant influence on the antimycobacterial activity of peptides. There is a need to optimize these parameters for the development of synthetic analogues with better selectivity of indices and increased synergistic potential with conventional agents [175].

S100A12 is small cationic protein (member of the S100 protein family), characterized by an EF-hand calcium binding motif. They take part in various intracellular and extracellular functions. Direct and indirect extracellular antimicrobial activity against filarial parasites, fungi, Gram-negative bacteria and Gram-positive bacteria has been attributed to S100A12 [186–189]. It has been suggested to have pro-inflammatory activity [190]. It is rich in 10-lysines, 1-arginine, and 6-histidines (cationic residues) and hydrophobic amino acids. Its amino acid composition has been quantitated and shown to follow the same trend of sequence as other known α-helical AMPs, which satisfy the criterion for negative Gaussian

curvature generation with membrane-permeating antimicrobial activity, such as LL-37 [191]. The ability of S100A12 to show direct activity against *M. tuberculosis* and *M. leprae* in axenic culture was investigated. With S100A12, there was a decrease in the mean colony forming units (CFUs) for the virulent *M. tuberculosis* (H37Ra) (CFUs = 4 × 103, P = 0.003; and CFUs = 1 × 103, P = 0.0009) at 0.1 and 1 μM of S100A12, when compared to the control (media alone, CFU = 23 × 103) [191]. The mean percent viability relative to the media alone was also determined, which showed a significant decrease (47% viability at 0.1 μM, P = 0.004) in the presence of S100A12 [191]. A decrease in the viability of *M. leprae* was also observed for each replicate in a dose-dependent manner. The antimicrobial activity was consistent with that of typical AMPs [191]. S100A12, as with other antimicrobial proteins, modulates host immune responses and activates the MAP-kinase and NF-κB pathways through the activation of RAGE and TLR4 [192,193]; as a result, it can elicit the secretion of cytokines and increase molecule adhesion in activated cells [194]. It is involved in other immunomodulatory activities, such as the chemotaxis of monocytes and the induction of cell migration [195]. During the analysis, S100A12 was linked to "chemotaxis" and "inflammatory response" (gene ontology terms), in addition to "defence response" and "killing the cells of other organisms" [191]. S100A12 protein has been implicated in several inflammatory diseases, such as inflammatory bowel disease, rheumatoid arthritis and psoriasis [196–198]; as a consequence, its level in the system has been suggested by many studies to be representative of disease states, as well as a clinical biomarker for inflammation. The specific role and function of S100A12 in these disease states are yet to be understood, despite been detected in the system and in the inflammatory compartments and tissues [191]. The detection of S100A12 in patients with RR and the ability to restrict infection, albeit with resultant nerve injury, indicate that the antimicrobial and inflammatory activity of these molecules is linked, as is the case with many AMPs [199,200].

8.2. β-sheet peptides (two or more disulfide bonds)

Human b-defensins (HBDs) have been reported as being part of the immune system [201], many of which have been found using computational approaches, applied to the human genome sequence [201], in order to demonstrate a wide range of antimicrobial activity [202–204]. The HBDs are cationic peptides comprising three antiparallel b-strands, reinforced by three conserved disulfide bridges [205], which offer a condensed small structure [206]. Many of them have an a-helix at the N-terminal region [207]. The main role of the HBDs is the management of the bacterial growth, either through influencing their membrane surface (HBD2 and HBD3) [87] or through acting in phagolysosomes (HBD1) [208]. Thus, the HBDs are described as endogenous antibiotics with a broad spectrum of bactericidal action, which can additionally suppress the growth and progress of fungi [206,209], protozoa and capsulated viruses [206,208–211]. It was reported that HBD2 showed antimicrobial activity against the *M. tuberculosis* strain H37Rv (1.5 μM). The HBD3-MHBD2 presented the best MIC value (2.7 μM) against MDR-TB, signifying the possibility of using the HBDs against pathogenic *M. tuberculosis* [212]. Santos et al. [213] reported that the magainin-I analogue peptide (MIAP), at a concentration of 300 μg/mL, showed an activity, which was two-fold higher when compared to the native peptide magainin-I with an MIC of 1200 μg/mL against *M. tuberculosis* [213]. A short synthetic peptide derivative from hBD3-Pep4, a human beta-defensin hBD3, was tested for activity against *M. smegmatis* [214]. The antimicrobial activity of peptide 4 of hBD3 was also determined [215] and found to be potent against *M. smegmatis* with an EC_{50} value of 0.36 μg/mL [214]. The observed activity was more than the reported values of other tested human beta-defensins. The activity of hBD2 of 1.5 μM against H37RV and 6.8 μM of hBD3- M-hBD2 was reported by Corrales-Garia et al. [212,216]. hBD-3 has been shown to offer the most potent range of antimicrobial effectiveness [214]. The beta-defensins demonstrates chemotactic properties in conjunction with their capability to directly initiate antimicrobial activity against invading pathogens, thereby forming a bridge innate between immunity and adaptive immunity [144,217,218]. Reports on the *in vivo* role of hBD3 against *Mycobacterium* spp. are still limited; however, many activities have been reported *in vitro* [216]. Among the tested AMPs for intracellular bactericidal activity, hBD3-Pep4 showed a remarkable activity against *M. smegmatis* compared to rifampicin and polymyxin B. However, the killing rate for hBD3-Pep4 and polymyxin B was 68% compared to 75% for polymyxin B and LL-37, rifampicin and LL-37, and rifampicin and CRAMP [214].

It has been proposed that MIAP intrudes with the H^+ pumping through the *M. tuberculosis* plasma membrane, F1F0-H^+ATPase. The suppression of this enzyme may be translated as an impaired capability to control the intracellular H^+ concentration (pHi) of the mycobacteria. The deregulation of pH is deleterious against bacteria. The obtained findings imply that AMPs, especially MIAP, in line with their direction, are capable of binding to the cytosolic and periplasmic sides of the mycobacterial plasma membrane. Furthermore, AMPs can interact with the mycobacteria membranes intruding in ATPase activity, particularly the F1F0-ATPase, which operates when the peptides are linked to the internal membrane side. Thus, an integrated impact on cell disturbance and inhibition of the ATPase activity of MIAP cannot be ignored [213].

Protegrin-1 (PG-1) is available in porcine neutrophils, where they can function in the non-oxidative activity of such cells. The mode through which the protegrins kill bacteria is uncertain, although their ability to disrupt microbial membranes is thought to be essential [163]. The PG-1 seems to form selective channels in the lipid membranes, which make up the bacterial lipopolysaccharide. The activities of two cysteine-rich APMs, specifically, the 18-residue beta-sheet PG-1 [219] and the 36-residue beta-sheet epithelial beta-defensin-1 (HBD-1) [220], have been evaluated against drug-susceptible or MDR-TB strains. The PG-1 was considerably active against the H37Rv strain, with a decrease in the CFU numbers at a concentration of 64 μg/mL (68.4%) and 128 μg/mL (96.7%), respectively.

Additionally, the HBD-1 was active against the H37Rv strain, with a considerable CFU decrease observed at a concentration of 128 μg/mL (49.9%). The MDR strain showed a greater resistance to both peptides in contrast to H37Rv, with a considerable CFU reduction only noted at a concentration of 128 μg/mL of PG-1 (45.1%) [217]. Considered as an antibacterial peptide, rich in proline and arginine, which is isolated from porcine leukocytes, PR-39 showed activity against drug-susceptible and MDR isolates of *M. tuberculosis*. The PR-39 showed an 80% growth inhibition in the H37Rv strain at 50 mg/L. The MDR-TB strains (E1380/94 and P34/95) were not susceptible to PR-39, with a growth inhibition of 39 and 49% at 50 mg/L of the peptide, respectively [114]. Azurophil granule proteins (AZPs) have been reported to be potent against mycobacteria. 100 mg/mL of AZPs was observed to kill approximately 55% of *M. tuberculosis* after 24 h of incubation, while a total killing of *M. smegmatis* was noted with 50 mg/mL of AZPs. The studies using electron microscopes have shown that AZPs kill mycobacteria by destroying their cell membrane [221].

The endogenous host defence peptides, as established components of innate immunity, have been proposed to have a significant function in TB infections. These peptides can immediately inhibit microbial growth through a range of membrane and non-membrane targets [222]. The immunomodulatory properties of IDR peptides include: (i) leukocyte activation; (ii) decreased pro-inflammatory responses to microbial products; (iii) macrophage

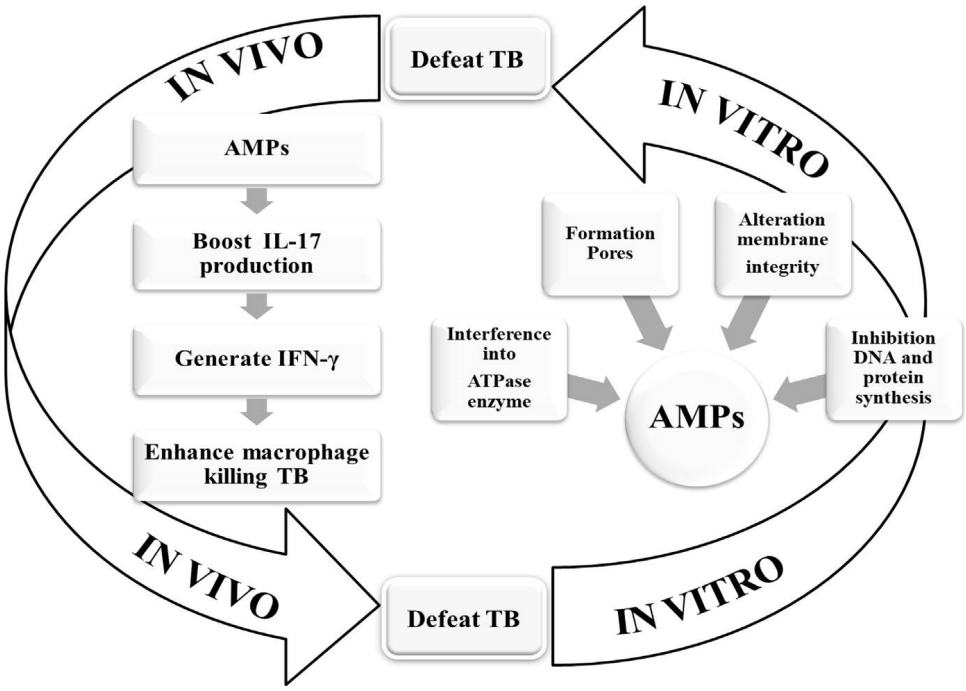

Fig. 4. How AMPs impact bacterial cells' survival *in vivo* and *in vitro*.

and leukocyte differentiation; and (iv) modulation of the expression of chemokines, reactive oxygen species and reactive nitrogen species [223]. The anti-infective operation of the three synthetic IDR (HH2, 1018 and 1002) peptides against *M. tuberculosis*, using drug-sensitive and MDR-TB strains, have been assessed. A 32 mg dose of each peptide in liquefied form, in 100 mL of a saline solution (1 mg/Kg), was administered intratracheally three times a week for a month. The HH2 (VQLRIRVAVIRA-NH2) and IDR-1018 (VRLIVAVRIWRR-NH2) peptides led to an adequate suppression of the tuberculosis bacilli growth and decreased the pneumonic region in an experimental model of pulmonary TB after 15 and 30 days of therapy, even though peptide 1002 did not show a considerable reduction in the bacillary loads. This occurred during the infection of the mice with the drug-resistant or drug-sensitive mycobacteria using a model with an elevated dose of infection in Balb/c mice, administered with the given treatment regime [224].

8.3. Others peptides

Peptoids (Oligo-N-replaced glycines) comprise sequence-particular peptidomimetics, which are based on a "biomimetic" peptide backbone comparable to a natural protein, while having their side chains attached to the amide nitrogen [225,226]. This variation in structure renders them greatly resistant to protease activity [227,228]. The 1-$C13_{4mer}$ showed a remarkable tuberculocidal action against *M. tuberculosis* with an MIC of 6.6 μM. Peptoid 1-$C13_{4mer}$ is cationic and four residues long, containing a 13-carbonaliphatic tail as part of its N-terminus. The hydrophobic tail of peptoid 1-$C13_{4mer}$ confers a surfactant-like nature and is anticipated to interact intensely and in a disruptive manner with the hydrophobic, waxy, lipid-enriched outer layer of mycobacterium, thereby facilitating a better peptoid permeation into the bacterium. The cationic surfactants are generally poisonous for mammalian cells and bacteria. In this case, however, the strong self-association of this oligopeptoid, imposed by its C-3 tail, has to protect macrophages, which, unlike bacteria, are not considerably anionic (or hydrophobic in the case of *M. tuberculosis*) on their outer membranes. The 1-$C13_{4mer}$ micellization at its MIC would be fully expected to enhance its anti-*M. tuberculosis* potency, as the labile micelles, following binding to the anionic or hydrophobic bacterial membrane, would detach, forming a high local concentration at the cell surface [229]. The mycobacterium cell membrane varies considerably from that of normal Gram-positive or Gram-negative bacteria. The exterior layer of the mycobacterium is made up of lipid-rich, hydrophobic layers of mycolic acid, as well as the aliphatic, hydrophobic wax barrier, which decreases the permeability of the *M. tuberculosis* cell membrane in relation to regular anti-tuberculosis drugs [230,231]. The ineffective permeation of the drugs and the trapping of the drugs by the waxy envelope layer partially accounts for the greater resistance of mycobacteria to antibiotics [232]. The slow metabolism of mycobacterium is additionally inclined to reduce the efficiency of most conventional agents, as the compounds normally function by damaging specific steps in the metabolic cycle of the cells.

ATRA-1A and ATRA-2, which are two synthetic 11-residue peptides with differences in the repeated motif within the Chinese cobra-derived cathelicidin NA-CATH, were tested for activity against *M. smegmatis*. As ATRA-1 has a higher potency compared to ATRA-2, the differences in the sequence may have influenced the activity of the peptide. ATRA-1A had an EC_{50} value of 0.05 μg/mL against *M. smegmatis*, while ATRA-2 caused no significant effect against *M. smegmatis* at any concentration [214]. This finding was consistent with previous reports in which the activity of ATRA-2 against most bacteria was found to be ineffective, due to amino acid substitution during its production, thereby making it a less potent AMP [233–236]. The helical structure of this peptide was likely tempered by the introduction of a proline at residue 11, thus interfering with its activity against microbes [233]. ATRA-1A had an EC_{50} of 0.035 μM against mycobacteria, making it the most potent identified antimycobacterial peptide to date [214]. Mouse cathelicidin (mCRAMP) had an EC50 of 0.17 μg/mL (0.044 μM) [198], a lower value compared to EC50 value of 58.81 μM against *M. avium*, as report by Carlos et al. [237], thereby suggesting a relevant physiological potency [237]. Moreover, mCRAMP showed the best MIC (15.6 μg/mL) among the peptides. Synergistic studies with rifampicin have only been reported for the full-length cathelicidins

Table 2
Examples of AMPs showing activity against *M. tuberculosis in vitro*/*in vivo* with respective modes of action.

Name	Structural classes	Activity	Proposed mode of action	Reference
D-V13 K (D5)	α-Helix	MIC (MDR-TB):15.6 μM MIC (*M. tuberculosis*):11.2 μM	Pore-formation	[152]
D-LAK analogues		MIC (*M. tuberculosis*): 50 μM MIC (MDR-TB): 100 μM	-Pore-formation -Inhibition of protein synthesis	[155]
Bacteriocins (Bcn1-Bcn5)		MIC (*M. tuberculosis*): 0.01–1 μg/mL	Pore-formation	[157,158]
Nisin A		MIC (*M. tuberculosis*): 60 μg/mL	-Pore-formation -Inhibition of cell wall biosynthesis	[160]
Lactoferrin		MIC (*M. tuberculosis*): 1 mg/mL *In vivo*: oral administration of 0.5% lactoferrin led to 1 log10 reduction in bacterial load.	Iron sequestration	[162]
LL37		*In vivo*: approximately 53% and 45% of *M. tuberculosis* and MDR-TB killing, respectively at 32 μg/mouse	Pore-formation	[144]
LLKKK18		*In vivo*: 1.2-log reduction at 100 μM of mice infected by *M. tuberculosis*	Pore formation	[89]
LLAP		MIC (*M. tuberculosis*): 600 μg/mL	Inhibition of the basal ATPase activity of the mycobacterial plasma membrane	[167]
S100A12		MIC (*M. tuberculosis*): 0.1 μM	Immunomodulatory activity	[201]
Human Beta Defensins (hBDs) variants	β-sheet	MIC (*M. tuberculosis*): 1.5 μM MIC (MDR-TB): 2.7 μM MIC (*M. smegmatis*): 0.36 μg/mL	Pore formation	[212]
Magainin-I analogue peptide (MIAP)		MIC (*M. tuberculosis*): 12–80 μg/mL	Inhibition of the ATPase activity	[213]
Protegrin-1 (PG-1)		MIC (*M. tuberculosis*): 64 – 128 μg/mL	Formation of cation-selective channels on bacterial membrane	[217]
PR-39		80% growth inhibition of *M. tuberculosis* at 50 mg/L. 39 and 49% growth inhibition of MDR-TB strains (E1380/94 and P34/95, respectively) at 50 mg/L	Inhibition of DNA and protein synthesis	[114]
Azurophil granule proteins (AZP)		Approximately 55% growth inhibition of *M. tuberculosis* at100 mg/mL MIC (*M. smegmatis*): 50 mg/mL	Destroying cell membrane	[221]
IDR-1018		*In vivo* (*M. tuberculosis* and MDR-infected mice): 10–71% killing at 32 μg/mouse	Immunomodulatory activity	[224]
1-$C13_{4mer}$	Others peptides	MIC (*M. tuberculosis*): 6.6 μM	Pore formation	[229]
ATRA-1A		MIC (*M. tuberculosis*): 0.035 μM EC_{50} value of 0.05 μg/mL against *M. smegmatis*	Membrane disruption	[214]
mCRAMP		EC_{50} value of 0.17 μg/mL against *M. avium*	Permeabilize membranes	[238]
Modified peptide-drug conjugate dpMtx		IC50 (*M. tuberculosis*): 950 nM IC50 (*M. smegmatis*): 160 nM	Membrane disruption	[240]

(mCRAMP) and never for the short synthetic peptides (ATRA-1A and hBD3-Pep4). A fractional inhibitory concentration index (FICI) score of 0.5 was reported when mCRAMP was conjugated with polymyxin B. A foundation for further studies may stem from these results concerning the activity of two small synthetic peptides against *M. smegmatis* [214]. They could also stimulate further studies on the development of potent antimycobacterial drugs against *M. tuberculosis* from these small peptides [238].

NA-CATH had an EC_{50} of 1.88 μg/mL, which is more than the EC50 value of ATRA-1A [214]. Previously, the haemolytic activities of ATRA-1A and NA-CATH have been reported [233]. There was no statistical difference in the haemolytic activity of NA-CATH and ATRA-1A when compared to PBS at pH 7 [236]. Similarly, no haemolytic activity was reported for mCRAMP [239].

This study deals with the synthesis and testing of a unique peptide-drug conjugate with a high level of activity against *M. smegmatis* and *M. tuberculosis*. It also covered the clearance of the intracellular mycobacteria from the cultured macrophages [240]. The unmodified drug methotrexate and the modified dpMtx were tested against two mycobacteria species for antimycobacterial activity [240]. The unmodified methotrexate at a concentration of 10 μM showed no activity against the tested organisms, but with a great toxicity towards RAW264.7 murine macrophages (IC50 = 24 nM). Meanwhile, significant antimycobacterial activity against *M. smegmatis* (IC50 = 160 nM) and *M. tuberculosis* (IC50 = 950 nM) was observed with the modified peptide-drug conjugate dpMtx. It also presented significantly less toxicity towards macrophages (IC50 = 9600 nM). The modified peptide-drug conjugate dpMtx cleared the intracellular *M. smegmatis* from the cultured macrophages in an untargeted manner [240]. The synergistic effect of mycobacteria co-treatment with dpMtx and other antibacterials was investigated in order to understand the mechanism of dpMtx action and evaluate its possible synergism with other antimicrobials, such as the first line *M. tuberculosis* treatment agents [241,242]. There was a synergistic action between dpMtx and sulfamethoxazole (folic acid synthesis inhibitor), as well as between cephalotaxine and ethambutol (cell wall synthesis inhibitor). With nalidixic acid, dpMtx showed no synergistic action, although an additive effect was observed with rifampicin (FIC = 0.625) and isoniazid (FIC = 0.75) [240]. The ability of dpMtx to traverse the mycolic acid cell wall and interact with dihydrofolate reductase suggests a synergistic combination with antifolates and cell wall inhibitors. This ability to synergize with cell wall inhibitors is interesting because the combination of antifolates and cell wall inhibitors could be a source of great combination therapy. From these data, it has been demonstrated that dpMtx is a potent antimycobacterial agent with a reduced cellular toxicity towards cultured macrophages [240]. Fig. 4 shows the possible mechanism of action

of AMPs against TB *in vitro* and *in vivo*. Moreover, some examples of AMPs displaying activity against *M. tuberculosis* with respective modes of action are listed in Table 2

9. Clinical trials

Several pharmaceutical companies in their search for novel antimicrobial agents have tried to introduce AMPs to the market [243]. Generally, current investigations focus on small and cost-efficient molecules, such as the biologically active core regions of the AMP. The AMPs are at different stages of drug development, from the pre-clinical to stage III of clinical studies, with many compounds involving various molecular sizes having been investigated. LTX-109, which is considered as a synthetic, membrane-degrading peptide, has been developed by Lytix Biopharma and is presently being subjected to phase II trials for MRSA nasal infection treatment [244]. Little success has been reported regarding the AMPs exposed to clinical trials, especially when the findings have been compared to the conventional antibiotics [174,245]. Otherwise promising AMPs, which are subject to clinical development, include surotomycin, a lipopeptide developed by Cubist Pharmaceuticals, which is in phase III trials for *Clostridium difficile* infection treatment [246].

MBI-226 (Omiganan), which has recently completed two different phase III clinical trials, has shown a considerable competence in reducing catheter colonization and decreasing microbiologically confirmed tunnel infections [247]. Plectasin®, isolated from the fungus *Pseudoplecta nianigrella* and the lantibiotic NAI-107, have presented a considerable level of competence towards MRSA [247,248]. Meanwhile, the endorsement of the AMP pexiganan, which is a wide-spectrum synthetic analogue from the African frog peptide magainin, developed for diabetic foot ulcer therapy, was declined for clinical use [243,249]. This compound was unable to show an edge over the currently available fluoroquinolone treatment [174,245]. HLF1-11, obtained from human lactoferrin, has attained the phase II stage of clinical trials for fungal and bacterial infection treatments [250]. MU1140, which has shown potency against MRSA and *Bacillus anthracis*, is an AMP that has completed pre-clinical trials for tuberculosis treatment following the observation of promising activity against *M. tuberculosis* [251,252].

10. Delivery systems for AMPs

Microgels are sparingly cross-linked colloidal gel particles, which can respond to a range of stimuli, such as changes in pH, temperature, ionic strength, redox conditions and the presence of specific metabolites, by suddenly undergoing a swelling transition. They are sometimes referred to as nanogels. Microgels can be formulated in order to be triggered by external fields, such as magnetic and light fields. They have attracted much interest in various areas, such as biomaterials, biosensors, advanced materials and drug delivery [253,254]. The loading of LLKKK18 (KEFKRIVKRIKKFLRKLV) onto hyaluronic acid nanogels has been studied by Silva et al. [255] with a view to achieving an efficient anti-tuberculosis effect. The incorporation of peptides into hyaluronic acid nanogels was found to enhance peptide stability and minimize cytotoxicity. Interestingly, nanogels have been found to be internalized effectively by macrophages; meanwhile, nanogel-loaded peptides were also co-localized with mycobacteria within host cells, resulting in a noticeable reduction in the number of mycobacteria in macrophages exposed to either *M. avium* or *M. tuberculosis*, as demonstrated by the reduced level of cytokine IL-6 and TNF-α. The infection levels in mice exposed to *M. avium* or *M. tuberculosis* were significantly reduced by the *in vivo* intra-tracheal administration of peptide-loaded nanogels after five to 10 days of administration [255].

Dry inhalation powders offer more convenience for storage and stability, compared to liquid aerosols, because of the reduced rate of biochemical degradation in solid states [256]. With dry inhalation powders, a rapid dose administration and high local drug deposition are possible since they are activated by patient-inspired effort, and have been an attractive strategy for antimicrobial formulations targeted against lung infections [257,258]. The maintenance of the physical integrity and stability of peptides during the drying process, while equally achieving good inhalability and aerodynamic properties for the powder, is the major challenge in preparing inhalable peptide-based therapeutic formulations [259].The production of inhalable dry powders composed of D-enantiomeric AMPs (D-LAK120-HP13 and D-LAK120-A) has been investigated by Lip Kwok et al. [259], along with the evaluation of their solid-state properties, structural conformation and aerosol performance. For the first time, the study demonstrated a successful formulation of these AMPs into dry powder formulations via spray-drying using mannitol as a bulking agent. The particle size of the spray-dried powder was desirable for inhalation and spherical in shape. The good aerosol performance of the powders also demonstrated its inhalability. Further physicochemical characterization of the spray-dried powders showed that mannitol crystallinity was retained, while the peptide's secondary structures were preserved during the process of spray-drying. There is potential to develop a new class of anti-tuberculosis agents from these inhalable powdered AMP formulations [259].

11. Outlook and conclusive remarks

Currently, TB is globally endemic and endangering the gains already made in its control. This is regardless of the introduction of inexpensive and efficient treatments using a combination of four drug regimens four decades ago. Novel drugs and enhanced means of assessing novel medications and regimens are urgently needed for shorter and less toxic TB treatment regimens. In the past 10 years, significant investments by financiers, scientists and the WHO have led to a renewal of activity into the evaluation and development of novel TB agents. Greater coordination and collaboration are urgently needed among drug developers and clinical trial networks as novel AMPs are currently undergoing clinical trials for bacterial and antifungal infection treatments. When combined with conventional anti-TB agents, AMPs could represent a potential strategy for TB treatment.

AMPs have been identified in plants, animals and single-celled organisms, involving a selective microbial action against a broad spectrum of organisms, mostly due to their electronic interaction with negatively charged membranes. Some AMPs also promote the host defence by modulating host cellular immunity in vertebrate species, while, in mammals, AMPs not only ward off pathogens, they also shape the composition of the microbiome, which is significant for the protection of many aspects of health. The growing problems associated with the resistance and abuse of conventional antibiotics have prompted interest in the development of AMPs as the next generation of anti-infective agents to combat pathogens. Regardless of the numerous limitations, the management of the generation of endogenous AMPs could represent the key paradigm in the treatment of a wide array of animal and human diseases.

Conflict of interest

The authors confirm that this article content has no conflict of interest.

References

[1] K. Elwood, Tuberculosis a comprehensive clinical reference, Clin. Infect. Dis. 50 (2010) 288.

[2] C. Dye, S. Scheele, P. Dolin, et al., Consensus statement. Global burden of tuberculosis: estimated incidence, prevalence, and mortality by in an urban community, Int. J. Tuberc. Lung Dis. 9 (2005) 970–976.
[3] R. Diel, R. Loddenkemper, J. Zellweger, et al., Old ideas to innovate TB control: preventive treatment to achieve elimination, Eur. Respir. J. 42 (2013) 785–801.
[4] M. Raviglione, B. Marais, K. Floyd, et al., Scaling up interventions to achieve global tuberculosis control: progress and new developments, Lancet 379 (2012) 1902–1913.
[5] Organization WH, World Health Organization Global Tuberculosis Report 2013, 2014.
[6] Organization WH, Global Tuberculosis Control: a Short Update Tothe 2009 Report, 2009.
[7] A. Hill, J. Becerra, K. Castro, Modelling tuberculosis trends in the USA, Epidemiol. Infect. 140 (2012) 1862–1872.
[8] A. Zumla, P. Nahid, S.T. Cole, Advances in the development of new tuberculosis drugs and treatment regimens, Nat. Rev. Drug Discov. 12 (2013) 388–404.
[9] A.A. Bahar, D. Ren, Antimicrobial peptides, Pharmaceuticals 6 (2013) 1543–1575.
[10] M. Pasupuleti, A. Schmidtchen, M. Malmsten, Antimicrobial peptides: key components of the innate immune system, Crit. Rev. Biotechnol. 32 (2012) 143–171.
[11] H. Zeya, J. Spitznagel, Cationic proteins of polymorphonuclear leukocyte lysosomes II. composition, properties, and mechanism of antibacterial action, J. Bacteriol. 91 (1966) 755–762.
[12] H. Zeya, J. Spitznagel, Arginine-rich proteins of polymorphonuclear leukocyte lysosomes antimicrobial specificity and biochemical heterogeneity, J. Exp. Med. 127 (1968) 927–941.
[13] M. Zasloff, Magainins A class of antimicrobial peptides from Xenopus skin: isolation, characterization of two active forms, and partial cDNA sequence of a precursor, Proc. Natl. Acad. Sci. U. S. A. 84 (1987) 5449–5453.
[14] R.I. Lehrer, W. Lu, α-Defensins in human innate immunity, Immunol. Rev. 245 (2012) 84–112.
[15] T. Nakatsuji, R.L. Gallo, Antimicrobial peptides: old molecules with new ideas, J. Invest. Dermatol. 132 (2012) 887–895.
[16] L. Tomasinsig, M. Zanetti, The cathelicidins-structure, function and evolution, Curr. Protein Pept. Sci. 6 (2005) 23–34.
[17] R. Kapoor, P.R. Eimerman, J.W. Hardy, et al., Efficacy of antimicrobial peptoids against Mycobacterium tuberculosis, Antimicrob. Agents Chemother. 55 (2011) 3058–3062.
[18] H.-J. Shin, Y.-S. Kwon, Treatment of drug susceptible pulmonary tuberculosis, Tuberc Respir Dis. 78 (2015) 161–167.
[19] A. Nunn, A. Jindani, D. Enarson, Results at 30 months of a randomised trial of two 8-month regimens for the treatment of tuberculosis, Int. J. Tuberc. Lung Dis. 15 (2011) 741–745.
[20] B.M.R. Councils, Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis: second report, Lancet 301 (1973) 1331–1339.
[21] B.M.R. Councils, Controlled clinical trial of four short-course (6-month) regimens of chemotherapy for treatment of pulmonary tuberculosis: third report, Lancet 304 (1974) 237–240.
[22] Organization WH, Treatment of Tuberculosis: Guidelines, World Health Organization, 2010.
[23] D. Falzon, E. Jaramillo, H. Schünemann, et al., WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update, Eur. Respir. J. 38 (2011) 516–528.
[24] L.V. Sacks, R.E. Behrman, Developing new drugs for the treatment of drug-resistant tuberculosis: a regulatory perspective, Tuberculosis 88 (2008) 93–100.
[25] A.M. Ginsberg, M. Spigelman, Challenges in tuberculosis drug research and development, Nature 13 (2007) 290–294.
[26] M.A. Arbex, M.D.C.L. Varella, H.R.D. Siqueira, et al., Antituberculosis drugs: drug interactions, adverse effects, and use in special situations-part 1: first-line drugs, J. Bras. Pneumol. 36 (2010) 626–640.
[27] M.B. Conde, J.R. Lapa e Silva, New regimens for reducing the duration of the treatment of drug-susceptible pulmonary tuberculosis, Drug Dev. Res. 72 (2011) 501–508.
[28] N.J. Garton, H. Christensen, Minnikin DE et al. Intracellular lipophilic inclusions of mycobacteria in vitro and in sputum, Microbiology 148 (2002) 2951–2958.
[29] L.G. Wayne, L.G. Hayes, An in vitro model for sequential study of shiftdown of Mycobacterium tuberculosis through two stages of nonreplicating persistence, Infect. Immun. 64 (1996) 2062–2069.
[30] R. Loebel, E. Shorr, H. Richardson, The influence of adverse conditions upon the respiratory metabolism and growth of human tubercle bacilli, J. Bacteriol. 26 (1933) 167.
[31] M.O. Shleeva, Y.K. Kudykina, G.N. Vostroknutova, et al., Dormant ovoid cells of Mycobacterium tuberculosis are formed in response to gradual external acidification, Tuberculosis 91 (2011) 146–154.
[32] G.V. Mukamolova, O. Turapov, J. Malkin, et al., Resuscitation-promoting factors reveal an occult population of tubercle bacilli in sputum, Am. J. Respir. Crit. Care Med. 181 (2010) 174–180.
[33] Y. Zhang, W.W. Yew, M.R. Barer, Targeting persisters for tuberculosis control, Antimicrob. Agents Chemother. 56 (2012) 2223–2230.
[34] I. Honeyborne, T.D. McHugh, I. Kuittinen, et al., Profiling persistent tubercule bacilli from patient sputa during therapy predicts early drug efficacy, BMC Med. 14 (2016) 1.
[35] T. Sullivan, Y.B. Amor, Global introduction of new multidrug-resistant tuberculosis drugs—balancing regulation with urgent patient needs, Emerg. Infect. Dis. 22 (2016).
[36] J.B. Nachega, R.E. Chaisson, Tuberculosis drug resistance: a global threat, Clin. Infect. Dis. 36 (2003) 24–30.
[37] J.P. Prasad, Conceptual Pharmacology, Universities Press, 2010.
[38] I. Abubakar, M. Zignol, D. Falzon, et al., Drug-resistant tuberculosis: time for visionary political leadership, Lancet Infect. Dis. 13 (2013) 529–539.
[39] L. London, H. Cox, F. Coomans, Multidrug-Resistant TB: Implementing the right to health through the right to enjoy the benefits of scientific progress, Health Hum. Rights J. 18 (2016) 25–41.
[40] H. McIlleron, G. Meintjes, W.J. Burman, et al., Complications of antiretroviral therapy in patients with tuberculosis: drug interactions, toxicity, and immune reconstitution inflammatory syndrome, J. Infect. Dis. 196 (2007) 63–75.
[41] D. Menzies, H. Al Jahdali, B. Al Otaibi, Recent developments in treatment of latent tuberculosis infection, Indian J. Med. Res. 133 (2011) 257–266.
[42] P. Sudre, G. Ten Dam, A. Kochi, Tuberculosis: a global overview of the situation today, Bull. World Health Organ. 70 (1992) 149.
[43] A. Zumla, R. Atun, M. Maeurer, et al., Viewpoint: scientific dogmas, paradoxes and mysteries of latent mycobacterium tuberculosis infection, Trop. Med. Int. Health 16 (2011) 79–83.
[44] K. Yuen, P. Woo, Tuberculosis in blood and marrow transplant recipients, Hematol. Oncol. 20 (2002) 51–62.
[45] C.E. Barry, H.I. Boshoff, V. Dartois, et al., The spectrum of latent tuberculosis: rethinking the biology and intervention strategies, Nat. Rev. Microbiol. 7 (2009) 845–855.
[46] K.A. Brogden, Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol. 3 (2005) 238–250.
[47] B.M. Peters, M.E. Shirtliff, M.A. Jabra-Rizk, Antimicrobial peptides: primeval molecules or future drugs? PLoS Pathog. 6 (2010) e1001067.
[48] M. Zasloff, Antimicrobial peptides of multicellular organisms, Nature 415 (2002) 389–395.
[49] R.E. Hancock, G. Diamond, The role of cationic antimicrobial peptides in innate host defences, Trends Microbiol. 8 (2000) 402–410.
[50] T. Ebenhan, O. Gheysens, H.G. Kruger, et al., Antimicrobial peptides: their role as infection-selective tracers for molecular imaging, BioMed Res. Int. 2014 (2014) 867381.
[51] A. Giuliani, G. Pirri, S. Nicoletto, Antimicrobial peptides: an overview of a promising class of therapeutics, Open Life Sci. 2 (2007) 1–33.
[52] A. Bahar, D. Ren, Antimicrobial peptides, Pharmaceuticals 6 (2013) 1543–1575.
[53] K. Midura-Nowaczek, A. Markowska, Antimicrobial peptides and their analogs: searching for new potential therapeutics, Perspect Medicin Chem. 6 (2014) 73.
[54] A. Rozek, C.L. Friedrich, R.E. Hancock, Structure of the bovine antimicrobial peptide indolicidin bound to dodecylphosphocholine and sodium dodecyl sulfate micelles, Biochemistry 39 (2000) 15765–15774.
[55] M. Pushpanathan, P. Gunasekaran, J. Rajendhran, Antimicrobial peptides: versatile biological properties, Int J Pept. 2013 (2013) 675391.
[56] M.H. Jindal, Mohd Le CF, M.Y. Yusof, et al., Net charge, hydrophobicity and specific amino acids contribute to the activity of antimicrobial peptides, JUMMEC 17 (2014).
[57] H.V. Westerhoff, D. Juretic, R.W. Hendler, M. Zasloff, Magainins and the disruption of membrane-linked free-energy transduction, Proc. Natl. Acad. Sci. U. S. A. 86 (1989) 6597–6601.
[58] C. Subbalakshmi, R. Nagaraj, N. Sitaram, Biological activities of c-terminal 15-residue synthetic fragment of melittin: design of an analog with improved antibacterial activity, FEBS Lett. 448 (1999) 62–66.
[59] Y. Park, S.C. Park, H.K. Park, et al., Structure-activity relationship of hp (2–20) analog peptide: enhanced antimicrobial activity by n-terminal random coil region deletion, Biopolymers 88 (2007) 199–207.
[60] M.L. Juba, D.K. Porter, E.H. Williams, et al., Helical cationic antimicrobial peptide length and its impact on membrane disruption, BBA Biomemb. 1848 (2015) 1081–1091.
[61] R. Bessalle, H. Haas, A. Goria, et al., Augmentation of the antibacterial activity of magainin by positive-charge chain extension, Antimicrob. Agents Chemother. 36 (1992) 313–317.
[62] M. Dathe, T. Wieprecht, H. Nikolenko, et al., Hydrophobicity, hydrophobic moment and angle subtended by charged residues modulate antibacterial and haemolytic activity of amphipathic helical peptides, FEBS Lett. 403 (1997) 208–212.
[63] A. Tossi, L. Sandri, A. Giangaspero, Amphipathic, α-helical antimicrobial peptides, Biopolymers 55 (2000) 4–30.
[64] K. Matsuzaki, O. Murase, N. Fujii, et al., An antimicrobial peptide, magainin 2, induced rapid flip-flop of phospholipids coupled with pore formation and peptide translocation, Biochemistry 35 (1996) 11361–11368.
[65] M.R. Yeaman, N.Y. Yount, Mechanisms of antimicrobial peptide action and resistance, Pharmacol. Rev. 55 (2003) 27–55.
[66] M. Dathe, H. Nikolenko, J. Meyer, et al., Optimization of the antimicrobial activity of magainin peptides by modification of charge, FEBS Lett. 501 (2001) 146–150.

[67] B. Mojsoska, H. Jenssen, Peptides and peptidomimetics for antimicrobial drug design, Pharmaceuticals 8 (2015) 366–415.
[68] L.M. Yin, M.A. Edwards, J. Li, et al., Roles of hydrophobicity and charge distribution of cationic antimicrobial peptides in peptide-membrane interactions, J. Biol. Chem. 287 (2012) 7738–7745.
[69] U. Sudheendra, V. Dhople, A. Datta, et al., Membrane disruptive antimicrobial activities of human β-defensin-3 analogs, Eur. J. Med. Chem. 91 (2015) 91–99.
[70] Z. Jiang, A.I. Vasil, J.D. Hale, R.E. Hancock, et al., Effects of net charge and the number of positively charged residues on the biological activity of amphipathic alpha-helical cationic antimicrobial peptides, Biopolymers 90 (2008) 369–383.
[71] A. Tossi, L. Sandri, A. Giangaspero, New consensus hydrophobicity scale extended to non-proteinogenic amino acids, Peptides 27 (2002) 416.
[72] J. Biro, Amino acid size, charge, hydropathy indices and matrices for protein structure analysis, Theor. Biol. Med. Model. 3 (2006) 1.
[73] A. Schmidtchen, M. Pasupuleti, M. Mörgelin, et al., Boosting antimicrobial peptides by hydrophobic oligopeptide end tags, J. Biol. Chem. 284 (2009) 17584–17594.
[74] N.Y. Yount, M.R. Yeaman, Immunocontinuum: perspectives in antimicrobial peptide mechanisms of action and resistance, Protein Pept. Lett. 12 (2005) 49–67.
[75] K.L. Gudiksen, I. Gitlin, D.T. Moustakas, et al., Increasing the net charge and decreasing the hydrophobicity of bovine carbonic anhydrase decreases the rate of denaturation with sodium dodecyl sulfate, Biophys. J. 91 (2006) 298–310.
[76] S. Rotem, A. Mor, Antimicrobial peptide mimics for improved therapeutic properties, BBA-Biomembranes 1788 (2009) 1582–1592.
[77] M. Zaman, E. Ahmad, A. Qadeer, et al., Nanoparticles in relation to peptide and protein aggregation, Int. J. Nanomedicine. 9 (2014) 899–912.
[78] Y. Chen, M.T. Guarnieri, A.I. Vasil, et al., Role of peptide hydrophobicity in the mechanism of action of α-helical antimicrobial peptides, Antimicrob. Agents Chemother. 51 (2007) 1398–1406.
[79] D. Phoenix, F. Harris, O. Daman, et al., The prediction of amphiphilic α-helices, Curr. Protein Pept. Sci. 3 (2002) 201–221.
[80] T. Wieprecht, M. Dathe, R.M. Epand, et al., Influence of the angle subtended by the positively charged helix face on the membrane activity of amphipathic, antibacterial peptides, Biochemistry 36 (1997) 12869–12880.
[81] N. Uematsu, K. Matsuzaki, Polar angle as a determinant of amphipathic α-helix-lipid interactions: a model peptide study, Biophys. J. 79 (2000) 2075–2083.
[82] L.S. Vermeer, Y. Lan, V. Abbate, et al., Conformational flexibility determines selectivity and antibacterial, antiplasmodial, and anticancer potency of cationic α-helical peptides, J. Biol. Chem. 287 (2012) 34120–34133.
[83] M. Dathe, T. Wieprecht, Structural features of helical antimicrobial peptides: their potential to modulate activity on model membranes and biological cells, BBA-Biomembr. 1462 (1999) 71–87.
[84] J.K. Lee, R. Gopal, S.-C. Park, et al., A proline-hinge alters the characteristics of the amphipathic α-helical AMPs, PLoS One 8 (2013) e67597.
[85] S.-A. Lee, Y.K. Kim, S.S. Lim, et al., Solution structure and cell selectivity of piscidin 1 and its analogues, Biochemistry 46 (2007) 3653–3663.
[86] Y. Xie, E. Fleming, J.L. Chen, et al., Effect of proline position on the antimicrobial mechanism of buforin II, Peptides 32 (2011) 677–682.
[87] K.J. Cutrona, B.A. Kaufman, D.M. Figueroa, et al., Role of arginine and lysine in the antimicrobial mechanism of histone-derived antimicrobial peptides, FEBS Lett. 589 (2015) 3915–3920.
[88] C.B. Park, K.-S. Yi, K. Matsuzaki, et al., Structure–activity analysis of buforin II, a histone H2A-derived antimicrobial peptide: the proline hinge is responsible for the cell-penetrating ability of buforin II, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 8245–8250.
[89] J. Silvaa, Antimicrobial Peptide Delivery from Self-assembling Hyaluronic Acid Nanoparticles for Tuberculosis Treatment, 2016.
[90] M. Fernandez-Vidal, S. Jayasinghe, A.S. Ladokhin, S.H. White, Folding amphipathic helices into membranes: amphiphilicity trumps hydrophobicity, J. Mol. Biol. 370 (2007) 459–470.
[91] Y. Chen, C.T. Mant, S.W. Farmer, R.E. Hancock, et al., Rational design of alpha-helical antimicrobial peptides with enhanced activities and specificity/therapeutic index, J. Biol. Chem. 280 (2005) 12316–12329.
[92] T. Ganz, M.E. Selsted, D. Szklarek, et al., Defensins. Natural peptide antibiotics of human neutrophils, J. Clin. Invest. 76 (1985) 1427.
[93] J.-R.C. García, A. Krause, S. Schulz, et al., Human β-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity, FASEB J. 15 (2001) 1819–1821.
[94] J. Harder, J. Bartels, E. Christophers, J.-M. Schröder, Isolation and characterization of human β-defensin-3, a novel human inducible peptide antibiotic, J. Biol. Chem. 276 (2001) 5707–5713.
[95] J. Harder, J. Bartels, E. Christophers, et al., A peptide antibiotic from human skin, Nature 387 (1997) 861.
[96] A.J. Ouellette, M.E. Selsted, Paneth cell defensins: endogenous peptide components of intestinal host defense, FASEB J. 10 (1996) 1280–1289.
[97] M. Zaiou, V. Nizet, R.L. Gallo, Antimicrobial and protease inhibitory functions of the human cathelicidin (hCAP18/LL-37) prosequence, J. Invest. Dermatol. 120 (2003) 810–816.
[98] R.M. Epand, H.J. Vogel, Diversity of antimicrobial peptides and their mechanisms of action, BBA-Biomembr. 1462 (1999) 11–28.
[99] G. Bocchinfuso, A. Palleschi, B. Orioni, et al., Different mechanisms of action of antimicrobial peptides: insights from fluorescence spectroscopy experiments and molecular dynamics simulations, J. Pept. Sci. 15 (2009) 550–558.
[100] A.J. Duplantier, M.L. Van Hoek, The human cathelicidin antimicrobial peptide LL-37 as a potential treatment for polymicrobial infected wounds, Front. Immunol. 4 (2013) 143.
[101] J. Carroll, L.A. Draper, P.M. O'Connor, et al., Comparison of the activities of the lantibiotics nisin and lacticin 3147 against clinically significant mycobacteria, Int. J. Antimicrob. Agents 36 (2010) 132–136.
[102] Y.-B. Li, Y.-Y. Xie, N.-N. Du, et al., Synthesis and in vitro antitubercular evaluation of novel sansanmycin derivatives, Bioorg. Med. Chem. Lett. 21 (2011) 6804–6807.
[103] A. Patrzykat, C.L. Friedrich, L. Zhang, et al., Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in Escherichia coli, Antimicrob. Agents Chemother. 46 (2002) 605–614.
[104] H. Sharma, R. Nagaraj, Human β-Defensin 4 with non-native disulfide bridges exhibit antimicrobial activity, PLoS One. 10 (2015) e0119525.
[105] C. Subbalakshmi, N. Sitaram, Mechanism of antimicrobial action of indolicidin, FEMS Microbiol. Lett. 160 (1998) 91–96.
[106] A. Ghosh, R.K. Kar, J. Jana, et al., Indolicidin targets duplex DNA: Structural and mechanistic insight through a combination of spectroscopy and microscopy, ChemMedChem 9 (2014) 2052–2058.
[107] G. Hao, Y.-H. Shi, Y.-L. Tang, et al., The intracellular mechanism of action on Escherichia coli of BF2-A/C, two analogues of the antimicrobial peptide Buforin 2, J. Microbiol. 51 (2013) 200–206.
[108] B.B. Finlay, R.E. Hancock, Can innate immunity be enhanced to treat microbial infections? Nat. Rev. Microbiol. 2 (2004) 497–504.
[109] N. Malanovic, K. Lohner, Antimicrobial peptides targeting gram-positive bacteria, Pharmaceuticals 9 (2016) 59.
[110] T. Cullen, W. Schofield, N. Barry, et al., Antimicrobial peptide resistance mediates resilience of prominent gut commensals during inflammation, Science 347 (2015) 170–175.
[111] A. Kalita, I. Verma, G. Khuller, Role of human neutrophil peptide-1 as a possible adjunct to antituberculosis chemotherapy, J. Infect. Dis. 190 (2004) 1476–1480.
[112] J.K. Rugutt, K.J. Rugutt, Antimycobacterial activity of steroids, long-chain alcohols and lytic peptides, Nat. Prod. Res. 26 (2012) 1004–1011.
[113] K. Takayama, C. Wang, G.S. Besra, Pathway to synthesis and processing of mycolic acids in Mycobacterium tuberculosis, Clin. Microbiol. Rev. 18 (2005) 81–101.
[114] C.M. Linde, S.E. Hoffner, E. Refai, et al., In vitro activity of PR-39, a proline-arginine-rich peptide, against susceptible and multi-drug-resistant Mycobacterium tuberculosis, J. Antimicrob. Chemother. 47 (2001) 575–580.
[115] J. Hu, M.J. Miller, Total synthesis of a mycobactin S, a siderophore and growth promoter of Mycobacterium smegmatis, and determination of its growth inhibitory activity against Mycobacterium tuberculosis, J. Am. Chem. Soc. 119 (1997) 3462–3468.
[116] A.K. Marr, W.J. Gooderham, R.E. Hancock, Antibacterial peptides for therapeutic use: obstacles and realistic outlook, Curr. Opin. Pharmacol. 6 (2006) 468–472.
[117] F. Reffuveille, C. de la Fuente-Núñez, S. Mansour, et al., A broad-spectrum antibiofilm peptide enhances antibiotic action against bacterial biofilms, Antimicrob. Agents Chemother. 58 (2014) 5363–5371.
[118] C. de la Fuente-Núñez, F. Reffuveille, C. Mansour Sarah, et al., D-Enantiomeric peptides that eradicate wild-type and multidrug-resistant biofilms and protect against lethal Pseudomonas aeruginosa infections, Chem. Biol. 22 (2015) 196–205.
[119] Z. Wang, C. de la Fuente-Núñez, Y. Shen, et al., Treatment of oral multispecies biofilms by an anti-biofilm peptide, PLoS One. 10 (2015) e0132512.
[120] S.M. Ribeiro, C. de la Fuente-Núñez, B. Baquir, et al., Antibiofilm peptides increase the susceptibility of carbapenemase-producing Klebsiella pneumoniae clinical isolates to β-lactam antibiotics, Antimicrob. Agents Chemother. 59 (2015) 3906–3912.
[121] F. Orlando, R. Ghiselli, O. Cirioni, et al., BMAP-28 improves the efficacy of vancomycin in rat models of gram-positive cocci ureteral stent infection, Peptides 29 (2008) 1118–1123.
[122] N.M. Mishra, Y. Briers, C. Lamberigts, et al., Evaluation of the antibacterial and antibiofilm activities of novel CRAMP–vancomycin conjugates with diverse linkers, Org. Biomol. Chem. 13 (2015) 7477–7486.
[123] X. Wu, Z. Li, X. Li, et al., Synergistic effects of antimicrobial peptide DP7 combined with antibiotics against multidrug-resistant bacteria, Drug Des. Dev. Ther. 11 (2017) 939.
[124] M. Hara, S.T. Housman, D.P. Nicolau, J.L. Kuti, In vitro pharmacodynamics of polymyxin B and tigecycline alone and in combination against carbapenem-resistant Acinetobacter baumannii, Antimicrob. Agents Chemother. 58 (2014) 874–879.
[125] E.B. Breidenstein, P. Courvalin, D. Meziane-Cherif, Antimicrobial activity of plectasin nz2114 in combination with cell wall targeting antibiotics against vana-type Enterococcus faecalis, Microb. Drug Resist. 21 (2015) 373–379.
[126] L.M. Phee, J.W. Betts, B. Bharathan, D.W. Wareham, Colistin and fusidic acid, a novel potent synergistic combination for treatment of multidrug-resistant Acinetobacter baumannii infections, Antimicrob. Agents Chemother. 59 (2015) 4544–4550.

[127] J. Jammal, F. Zaknoon, G. Kaneti, K. Goldberg, A. Mor, Sensitization of Gram-negative bacteria to rifampin and OAK combinations, Sci. Rep. 5 (2015) 9216.
[128] O. Tabbene, S. Azaiez, A. Di Grazia, et al., Bacillomycin D and its combination with amphotericin B: promising antifungal compounds with powerful antibiofilm activity and wound-healing potency, J. Appl. Microbiol. 120 (2016) 289–300.
[129] E.Y. Kım, G. Rajasekaran, S.Y. Shın, LL-37-derived short antimicrobial peptide KR-12-a5 and its d-amino acid substituted analogs with cell selectivity, anti-biofilm activity, synergistic effect with conventional antibiotics, and anti-inflammatory activity, Eur. J. Med. Chem. 136 (2017) 428–441.
[130] A. Lewıes, J.F. Wentzel, A. Jordaan, et al., Interactions of the antimicrobial peptide nisin Z with conventional antibiotics and the use of nanostructured lipid carriers to enhance antimicrobial activity, Int. J. Pharm. 526 (2017) 244–253.
[131] M.F. Mohamed, A. Abdelkhalek, M.N. Seleem, Evaluation of short synthetic antimicrobial peptides for treatment of drug-resistant and intracellular Staphylococcus aureus, Sci. Rep. 6 (2016) 29707.
[132] K. Ogata, B.A. Linzer, R.I. Zuberi, et al., Activity of defensins from human neutrophilic granulocytes against Mycobacterium avium-Mycobacterium intracellulare, Infect. Immun. 60 (1992) 4720–4725.
[133] S. Sharma, I. Verma, G.K. Khuller, Therapeutic potential of human neutrophil peptide 1 against experimental tuberculosis, Antimicrob. Agents Chemother. 45 (2001) 639–640.
[134] Y. Miyakawa, P. Ratnakar, A.G. Rao, et al., In vitro activity of the antimicrobial peptides human and rabbit defensins and porcine leukocyte protegrin against Mycobacterium tuberculosis, Infect. Immun. 64 (1996) 926–932.
[135] J.F. Gera, A. Lichtenstein, Human neutrophil peptide defensins induce single strand DNA Breaks in target cells, Cell. Immunol. 138 (1991) 108–120.
[136] S. Sharma, G. Khuller, DNA as the intracellular secondary target for antibacterial action of human neutrophil peptide-I against mycobacterium tuberculosis H37Ra, Curr. Microbiol. 43 (2001) 74–76.
[137] G.A. Rook, J. Steele, L. Fraher, et al., Vitamin D3, gamma interferon, and control of proliferation of Mycobacterium tuberculosis by human monocytes, Immunology 57 (1986) 159–163.
[138] A.J. Crowle, E.J. Ross, Inhibition by retinoic acid of multiplication of virulent tubercle bacilli in cultured human macrophages, Infect. Immun. 57 (1989) 840–844.
[139] P.T. Liu, S. Stenger, H. Li, L. Wenzel, et al., Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response, Science 311 (2006) 1770–1773.
[140] S. Thoma-Uszynski, S. Stenger, O. Takeuchi, et al., Induction of direct antimicrobial activity through mammalian toll-like receptors, Science 291 (2001) 1544–1547.
[141] P.T. Liu, S. Stenger, D.H. Tang, et al., Cutting edge: vitamin D-mediated human antimicrobial activity against Mycobacterium tuberculosis is dependent on the induction of cathelicidin, J. Immunol. 179 (2007) 2060–2063.
[142] E. Sato, S. Imafuku, K. Ishii, et al., Vitamin D-dependent cathelicidin inhibits Mycobacterium marinum infection in human monocytic cells, J. Dermatol. Sci. 70 (2013) 166–172.
[143] J.M. Yuk, D.M. Shin, H.M. Lee, et al., Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin, Cell Host Microbe. 6 (2009) 231–243.
[144] B. Rivas-Santiago, C.E.R. Santiago, J.E. Castañeda-Delgado, et al., Activity of LL-37, CRAMP and antimicrobial peptide-derived compounds E2, E6 and CP26 against Mycobacterium tuberculosis, Int. J. Antimicrob. Agents 41 (2013) 143–148.
[145] H.G. Boman, B. Agerberth, A. Boman, Mechanisms of action on Escherichia coli of cecropin P1 and PR-39, two antibacterial peptides from pig intestine, Infect. Immun. 61 (1993) 2978–2984.
[146] A.E. Kieffer, Y. Goumon, O. Ruh, et al., The N- and C-terminal fragments of ubiquitin are important for the antimicrobial activities, FASEB J. 17 (2003) 776–778.
[147] S. Alonso, K. Pethe, D.G. Russell, et al., Lysosomal killing of Mycobacterium mediated by ubiquitin-derived peptides is enhanced by autophagy, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 6031–6036.
[148] G.E. Purdy, D.G. Russell, Lysosomal ubiquitin and the demise of Mycobacterium tuberculosis, Cell. Microbiol. 9 (2007) 2768–2774.
[149] G.E. Purdy, M. Niederweis, D.G. Russell, Decreased outer membrane permeability protects mycobacteria from killing by ubiquitin-derived peptides, Mol. Microbiol. 73 (2009) 844–857.
[150] M.H. Foss, K.M. Powers, G.E. Purdy, Structural and functional characterization of mycobactericidal ubiquitin-derived peptides in model and bacterial membranes, Biochemistry 51 (2012) 9922–9929.
[151] A. Sharma, A.A. Pohane, S. Bansal, et al., Cell penetrating synthetic antimicrobial peptides (SAMPs) exhibiting potent and selective killing ofmycobacterium by targeting its DNA, Chemistry 21 (2015) 3540–3545.
[152] Z. Jiang, P.M. Higgins, J. Whitehurst, et al., Anti-tuberculosis activity of α-helical antimicrobial peptides: de novo designed L-and D-Enantiomers versus L-and D-LL37, Protein Pept. Lett. 18 (2011) 241–252.
[153] S.Y. Hong, J.E. Oh, K.-H. Lee, Effect of D-amino acid substitution on the stability, the secondary structure, and the activity of membrane-active peptide, Biochem. Pharmacol. 58 (1999) 1775–1780.
[154] P. Grieco, A. Carotenuto, L. Auriemma, et al., The effect of d-amino acid substitution on the selectivity of temporin L towards target cells: identification of a potent anti-Candida peptide, BBA-Biomembr. 2013 (1828) 652–660.
[155] Y. Lan, J.T. Lam, G.K. Siu, et al., Cationic amphipathic D-enantiomeric antimicrobial peptides with in vitro and ex vivo activity against drug-resistant Mycobacterium tuberculosis, Tuberculosis 94 (2014) 678–689.
[156] J.S. Khara, Y. Wang, X.-Y. Ke, et al., Anti-mycobacterial activities of synthetic cationic α-helical peptides and their synergism with rifampicin, Biomaterials 35 (2014) 2032–2038.
[157] C. Chatterjee, M. Paul, L. Xie, et al., Biosynthesis and mode of action of lantibiotics, Chem. Rev. 105 (2005) 633–684.
[158] C. Guinane, P. Cotter, C. Hill, et al., Microbial solutions to microbial problems; lactococcal bacteriocins for the control of undesirable biota in food, J. Appl. Microbiol. 98 (2005) 1316–1325.
[159] V. Sosunov, V. Mischenko, B. Eruslanov, et al., Antimycobacterial activity of bacteriocins and their complexes with liposomes, J. Antimicrob. Chemother. 59 (2007) 919–925.
[160] J. Carroll, D. Field, P.M. O'Connor, et al., The gene encoded antimicrobial peptides, a template for the design of novel anti-mycobacterial drugs, Bioeng. Bugs. 1 (2010) 408–412.
[161] J.K. Actor, S.-A. Hwang, M.L. Kruzel, Lactoferrin as a natural immune modulator, Curr. Pharm. Des. 15 (2009) 1956–1973.
[162] K.J. Welsh, S.-A. Hwang, S. Boyd, et al., Influence of oral lactoferrin on Mycobacterium tuberculosis induced immunopathology, Tuberculosis 91 (2011) 105–113.
[163] A.R. Martineau, S.M. Newton, K.A. Wilkinson, et al., Neutrophil-mediated innate immune resistance to mycobacteria, J. Clin. Invest. 117 (2007) 1988–1994.
[164] K.D. Roby, A.D. Nardo, Innate immunity and the role of the antimicrobial peptide cathelicidin in inflammatory skin disease, Drug Discov. Today Dis. Mech. 10 (2013) 79–82.
[165] Y. De, Q. Chen, A.P. Schmidt, et al., LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells, J. Exp. Med. 192 (2000) 1069–1074.
[166] S. Gupta, K. Winglee, R. Gallo, et al., Bacterial subversion of cAMP signalling inhibits cathelicidin expression, which is required for innate resistance to Mycobacterium tuberculosis, J. Pathol. 242 (2017) 52–61.
[167] S. Chingaté, G. Delgado, L.M. Salazar, et al., The ATPase activity of the mycobacterial plasma membrane is inhibited by the LL37-analogous peptide LLAP, Peptides 71 (2015) 222–228.
[168] H.T. Chou, H.W. Wen, T.Y. Kuo, et al., Interaction of cationic antimicrobial peptides with phospholipid vesicles and their antibacterial activity, Peptides 31 (2010) 1811–1820.
[169] S. Lata, B.K. Sharma, G.P.S. Raghava, Analysis and prediction of antibacterial peptides, BMC Bioinf. 8 (2007) 263.
[170] G. Wang, B. Mishra, R.F. Epand, et al., High-quality 3D structures shine light on antibacterial, anti-biofilm and antiviral activities of human cathelicidin LL-37 and its fragments, Biochim. Biophys. Acta –Biomembr. 2014 (1838) 2160–2172.
[171] P. Chène, ATPases as drug targets: learning from their structure, Nat. Rev. Drug Discov. 1 (2002) 665–673.
[172] A. Zumla, M. Raviglione, R. Hafner, et al., Tuberculosis, N. Engl. J. Med. 368 (2013) 745–755.
[173] K.E.A. Lougheed, D.L. Taylor, S.A. Osborne, et al., New anti tuberculosis agents amongst known drugs, Tuberculosis 89 (2009) 364–370.
[174] C.D. Fjell, J.A. Hiss, R.E. Hancock, et al., Designing antimicrobial peptides: form follows function, Nat. Rev. Drug Discov. 11 (2012) 37–51.
[175] J.S. Khara, F.K. Lim, Y. Wang, et al., Designing α-helical peptides with enhanced synergism and selectivity against Mycobacterium smegmatis: discerning the role of hydrophobicity and helicity, Acta Biomater. 28 (2015) 99–108.
[176] M.P. Dos Santos Cabrera, M. Arcisio-Miranda, S.T. Broggio Costa, et al., Study of the mechanism of action of anoplin, a helical antimicrobial decapeptide with ion channel-like activity, and the role of the amidated C-terminus, J. Pept. Sci. 14 (2008) 661–669.
[177] L.T. Nguyen, J.K. Chau, N.A. Perry, et al., Serum stabilities of short tryptophan-and arginine-rich antimicrobial peptide analogs, PLoS One 5 (2010) 12684.
[178] Z. Jiang, B.J. Kullberg, H. Van Der Lee, et al., Effects of hydrophobicity on the antifungal activity of á –helical antimicrobial peptides, Chem. Biol. Drug Des. 72 (2008) 483–495.
[179] S.S. Zimmerman, H.A. Scheraga, Influence of local interactions on protein structure. I. Conformational energy studies of N-acetyl-N'-methylamides of Pro-X and X-Pro dipeptides, Biopolymers 16 (1977) 811–843.
[180] L. Piela, G. Némethy, H.A. Scheraga, Proline-induced constraints in á –helices, Biopolymers 26 (1987) 1587–1600.
[181] S. Kumar, M. Bansal, Dissecting á –helices: position-specific analysis of á –helices in globular proteins, Proteins 31 (1998) 460–476.
[182] M.K. Kim, Y.K. Kang, Positional preference of proline in á –helices, Protein Sci. 8 (1999) 1492–1499.
[183] Y. Chen, C.T. Mant, S.W. Farmer, et al., Rational design of á –helical antimicrobial peptides with enhanced activities and specificity/therapeutic index, J. Biol. Chem. 280 (2005) 12316–12329.
[184] A. Giangaspero, L. Sandri, A. Tossi, Amphipathic á helical antimicrobial peptides, Eur. J. Biochem. 268 (2001) 5589–5600.

[185] B. Jacob, I.S. Park, J.K. Bang, S.Y. Shin, Short KR-12 analogs designed from human cathelicidin LL-37 possessing both antimicrobial and antiendotoxic activities without mammalian cell toxicity, J. Pept. Sci. 19 (2013) 700–707.
[186] J.D. Gottsch, S.W. Eisinger, S.H. Liu, et al., Calgranulin C has filariacidal and filariastatic activity, Infect. Immun. 67 (1999) 6631–6636.
[187] M.H. Kim, Y.W. Choi, H.Y. Choi, et al., The expression of RAGE and EN-RAGE in leprosy, Br. J. Dermatol. 154 (2006) 594–601.
[188] K.P. Haley, A.G. Delgado, M.B. Piazuelo, B.L. Mortensen, P. Correa, S.M. Damo, et al., The human antimicrobial protein calgranulin C participates in control of helicobacter pylori growth and regulation of virulence, Infect. Immun. 83 (2015) 2944–2956.
[189] L.S. Cunden, A. Gaillard, E.M. Nolan, Calcium ions tune the zinc-Sequestering properties and antimicrobial activity of human S100A12, Chem. Sci. 7 (2016) 1338–1348.
[190] J. Pietzsch, S. Hoppmann, Human S100A12: a novel key player in inflammation? Amino Acids 36 (2009) 381–389.
[191] S. Realegeno, K.M. Kelly-Scumpia, A.T. Dang, et al., S100A12 is part of the antimicrobial network against mycobacterium leprae in human macrophages, PLoS Pathog. 12 (2016) 1005705.
[192] D. Foell, H. Wittkowski, C. Kessel, et al., Proinflammatory S100A12 can activate human monocytes via Toll-like receptor 4, Am. J. Respir. Crit. Care Med. 187 (2013) 1324–1334.
[193] M.A. Hofmann, S. Drury, C. Fu, et al., RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides, Cell 97 (1999) 889–901.
[194] P.K. Rouleau Vandal, C. Ryckman, et al., The calcium-binding protein S100A12 induces neutrophil adhesion, migration, and release from bone marrow in mouse at concentrations similar to those found in human inflammatory arthritis, Clin. Immunol. 107 (2003) 46–54.
[195] Z. Yang, T. Tao, M.J. Raftery, et al., Proinflammatory properties of the human S100 protein S100A12, J. Leukoc. Biol. 69 (2001) 986–994.
[196] D. Foell, D. Kane, B. Bresnihan, et al., Expression of the pro-inflammatory protein S100A12 (EN-RAGE) in rheumatoid and psoriatic arthritis, Rheumatology (Oxford) 42 (2003) 1383–1389.
[197] D. Foell, T. Kucharzik, M. Kraft, T. Vogl, C. Sorg, W. Domschke, et al., Neutrophil derived human S100A12(EN-RAGE) is strongly expressed during chronic active inflammatory bowel disease, Gut 52 (2003) 847–853.
[198] A. Mirmohammadsadegh, E. Tschakarjan, A. Ljoljic, et al., Calgranulin C is overexpressed in lesional psoriasis, J. Invest. Dermatol. 114 (2000) 1207–1208.
[199] J.P. Zackular, W.J. Chazin, E.P. Skaar, Nutritional immunity: s100 proteins at the host-Pathogen interface, J. Biol. Chem. 290 (2015) 18991–18998.
[200] Y. Lai, R.L. Gallo, AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense, Trends Immunol. 30 (2009) 131–141.
[201] B.C. Schutte, J.P. Mitros, Bartlett JA, et al. Discovery of five conserved β-defensin gene clusters using a computational search strategy, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2129–2133.
[202] W. Song, Y. Shi, M. Xiao, et al., In vitro bactericidal activity of recombinant human β-defensin-3 against pathogenic bacterial strains in human tooth root canal, Int. J. Antimicrob. Agents 33 (2009) 237–243.
[203] T. Ganz, Versatile defensins, Science 298 (2002) 977–979.
[204] T. Ganz, The role of antimicrobial peptides in innate immunity, Integr. Comp. Biol. 43 (2003) 300–304.
[205] E. Klüver, S. Schulz-Maronde, S. Scheid, et al., Structure-activity relation of human β-defensin 3: influence of disulfide bonds and cysteine substitution on antimicrobial activity and cytotoxicity, Biochemistry 44 (2005) 9804–9816.
[206] J.J. Schneider, A. Unholzer, M. Schaller, et al., Human defensins, J. Mol. Med. 83 (2005) 587–595.
[207] D.M. Hoover, Z. Wu, K. Tucker, et al., Antimicrobial characterization of human β-defensin 3 derivatives, Antimicrob. Agents Chemother. 47 (2003) 2804–2809.
[208] J.-M. Schröder, J. Harder, Human beta-defensin-2, Int. J. Biochem. Cell Biol. 31 (1999) 645–651.
[209] M. Schwaab, S. Hansen, M. Pearson, et al., Human β-defensins—at the front line of the peritonsillar abscess, Eur. J. Clin. Microbiol. Infect. Dis. 28 (2009) 745–755.
[210] R.I. Lehrer, T. Ganz, Defensins of vertebrate animals, Curr. Opin. Immunol. 14 (2002) 96–102.
[211] M. Mathews, H.P. Jia, J.M. Guthmiller, et al., Production of β-defensin antimicrobial peptides by the oral mucosa and salivary glands, Infect. Immun. 67 (1999) 2740–2745.
[212] L. Corrales-Garcia, E. Ortiz, J. Castañeda-Delgado, et al., Bacterial expression and antibiotic activities of recombinant variants of human β-defensins on pathogenic bacteria and M. tuberculosis, Protein Expr. Purif. 89 (2013) 33–43.
[213] P. Santos, A. Gordillo, L. Osses, et al., Effect of antimicrobial peptides on ATPase activity and proton pumping in plasma membrane vesicles obtained from mycobacteria, Peptides 36 (2012) 121–128.
[214] K. Gupta, S. Singh, M.L. van Hoek Short, Synthetic cationic peptides have antibacterial activity against mycobacterium smegmatis by forming pores in membrane and synergizing with antibiotics, Antibiotics (Basel) 4 (2015) 358–378.
[215] E.A. Papanastasiou, Q. Hua, A. Sandouk, et al., Role of acetylation and charge in antimicrobial peptides based on human beta-defensin-3, Acta Pathol. Microbiol. Immunol. Scand. 117 (2009) 492–499.
[216] J.I. Castaneda-Sanchez, B.E. Garcia-Perez, Munoz-Duarte, et al., Defensin production by human limbo-corneal Fibroblasts infected with Mycobacteria, Pathogens 2 (2013) 13–32.
[217] L. Fattorini, R. Gennaro, M. Zanetti, et al., In vitro activity of protegrin-1 and beta-defensin-1, alone and in combination with isoniazid, against Mycobacterium tuberculosis, Peptides 25 (2004) 1075–1077.
[218] D. Yang, A. Biragyn, L.W. Kwak, et al., Mammalian defensins in immunity: more than just microbicidal, Trends Immunol. 23 (2002) 291–296.
[219] R. Gennaro, M. Zanetti, Structural features and biological activities of the cathelicidin-derived antimicrobial peptides, Pept. Sci. 55 (2000) 31–49.
[220] D.M. Hoover, O. Chertov, J. Lubkowski, The Structure of human β-defensin-1 new insights into structural properties of β-defensins, J. Biol. Chem. 276 (2001) 39021–39026.
[221] P. Jena, S. Mohanty, T. Mohanty, et al., Azurophil granule proteins constitute the major mycobactericidal proteins in human neutrophils and enhance the killing of mycobacteria in macrophages, PLoS One 7 (2012) e50345.
[222] R.E. Hancock, H.-G. Sahl, Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies, Nat. Biotechnol. 24 (2006) 1551–1557.
[223] A.L. Hilchie, K. Wuerth, R.E. Hancock, Immune modulation by multifaceted cationic host defense (antimicrobial) peptides, Nat. Chem. Biol. 9 (2013) 761–768.
[224] B. Rivas-Santiago, J.E. Castañeda-Delgado, C.E.R. Santiago, et al., Ability of innate defence regulator peptides IDR-1002, IDR-HH2 and IDR-1018 to protect against Mycobacterium tuberculosis infections in animal models, PLoS One 8 (2013) e59119.
[225] J.A. Patch, A.E. Barron, Mimicry of bioactive peptides via non-natural, sequence-specific peptidomimetic oligomers, Curr. Opin. Chem. Biol. 6 (2002) 872–877.
[226] R.N. Zuckermann, J.M. Kerr, S.B. Kent, et al., Efficient method for the preparation of peptoids [oligo (N-substituted glycines)] by submonomer solid-phase synthesis, J. Am. Chem. Soc. 114 (1992) 10646–10647.
[227] K. Kirshenbaum, A.E. Barron, R.A. Goldsmith, et al., Sequence-specific polypeptoids: a diverse family of heteropolymers with stable secondary structure, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 4303–4308.
[228] S.M. Miller, R.J. Simon, S. Ng, et al., Comparison of the proteolytic susceptibilities of homologous L-amino acid, D-amino acid, and N-substituted glycine peptide and peptoid oligomers, Drug Dev. Res. 35 (1995) 20–32.
[229] Y. Shai, Mode of action of membrane active antimicrobial peptides, Pept. Sci. 66 (2002) 236–248.
[230] P.J. Brennan, H. Nikaido, The envelope of mycobacteria, Annu. Rev. Biochem. 64 (1995) 29–63.
[231] R. Johnson, Understanding the Mechanisms of Drug Resistance in Enhancing Rapid Molecular Detection of Drug Resistance in Mycobacterium Tuberculosis PhD Thesis, University of Stellenbosch, 2007.
[232] P. Méndez-Samperio, Role of antimicrobial peptides in host defense against mycobacterial infections, Peptides 29 (2008) 1836–1841.
[233] L.S. Amer, et al., Antimicrobial activity of the Naja atra cathelicidin and related small peptides, Biochem. Biophys. Res. Commun. 396 (2010) 825–830.
[234] L.S. Amer, B.M. Bishop, M.L. van Hoek, Antimicrobial and antibiofilm activity of cathelicidins and short, synthetic peptides against Francisella, Biochem. Biophys. Res. Commun. 396 (2010) 246–251.
[235] S.N. Dean, B.M. Bishop, M.L. van Hoek, Susceptibility of Pseudomonas aeruginosa biofilm to alpha-helical peptides: d-enantiomer of LL-37, Front. Microbiol. 2 (2011) e128.
[236] S.N. Dean, B.M. Bishop, M.L. van Hoek, Natural and synthetic cathelicidin peptides with anti-microbial and anti-biofilm activity against Staphylococcus aureus, BMC Microbiol. 11 (2011) e114.
[237] J.C. Santos, S. Silva-Gomes, Silva JP, et al. Endogenous cathelicidin production limits inflammation and protective immunity to Mycobacterium avium in mice, Immun. Inflamm. Dis. 2 (2014) 1–12.
[238] S. Ramon-Garcia, R. Mikut, C. Ng, et al., Targeting Mycobacterium tuberculosis and other microbial pathogens using improved synthetic antibacterial peptides, Antimicrob. Agents Chemother. 57 (2013) 2295–2303.
[239] S.W. Kang, D.G. Lee, S.T. Yang, et al., CRAMP analog having potent antibiotic activity without hemolytic activity, Protein Pept. Lett. 9 (2002) 275–282.
[240] S.O. Kelley, M.P. Pereira, J. Shi, Peptide targeting of an antibiotic prodrug toward phagosome-Entrapped mycobacteria, ACS Infect Dis. 1 (2015) 586–592.
[241] M.A. Farha, E.D. Brown, Chemical probes of Escherichia coli uncovered through chemical–chemical interaction profiling with compounds of known biological activity, Chem. Biol. 17 (2010) 852–862.
[242] S. Zlitni, L.F. Ferruccio, E.D. Brown, Metabolic suppression identifies new antibacterial inhibitors under nutrient limitation, Nat. Chem. Biol. 9 (2013) 796–804.
[243] S.-J. Kang, D.-H. Kim, T. Mishig-Ochir, et al., Antimicrobial peptides: their physicochemical properties and therapeutic application, Arch. Pharm. Res. 35 (2012) 409–413.
[244] L.D. Saravolatz, J. Pawlak, L. Johnson, et al., In vitro activities of LTX-109, a synthetic antimicrobial peptide, against methicillin-resistant, vancomycin-intermediate, vancomycin-resistant, daptomycin-nonsusceptible, and linezolid-nonsusceptible Staphylococcus aureus, Antimicrob. Agents Chemother. 56 (2012) 4478–4482.

[245] J.N. Afacan, T.Y.A. Yeung, M.O. Pena, et al., Therapeutic potential of host defense peptides in antibiotic-resistant infections, Curr. Pharm. Des. 18 (2012) 807–819.
[246] J.L. Fox, Antimicrobial peptides stage a comeback, Nat. Biotechnol. 31 (2013) 379–382.
[247] E. Rubinchik, D. Dugourd, T. Algara, et al., Antimicrobial and antifungal activities of a novel cationic antimicrobial peptide, omiganan, in experimental skin colonisation models, Int. J. Antimicrob. Agents 34 (2009) 457–461.
[248] D. Jabés, C. Brunati, G. Candiani, et al., Efficacy of the new lantibiotic NAI-107 in experimental infections induced by multidrug-resistant Gram-positive pathogens, Antimicrob. Agents Chemother. 55 (2011) 1671–1676.
[249] Y. Wang, D.E. Schlamadinger, J.E. Kim, et al., Comparative molecular dynamics simulations of the antimicrobial peptide CM15 in model lipid bilayers, BBA-Biomembr. 2012 (1818) 1402–1409.
[250] A.M. van der Does, P.J. Hensbergen, S.J. Bogaards, et al., The human lactoferrin-derived peptide hLF1-11 exerts immunomodulatory effects by specific inhibition of myeloperoxidase activity, J. Immunol. 188 (2012) 5012–5019.
[251] O. Ghobrial, H. Derendorf, J.D. Hillman, Pharmacokinetic and pharmacodynamic evaluation of the lantibiotic MU1140, J. Pharm. Sci. 99 (2010) 2521–2528.
[252] A. Padhi, M. Sengupta, S. Sengupta, et al., Antimicrobial peptides and proteins in mycobacterial therapy: current status and future prospects, Tuberculosis 94 (2014) 363–373.
[253] M. Malmsten, H. Bysell, P. Hansson, Biomacromolecules in microgels –opportunities and challenges for drug delivery, Curr. Opin. Colloid Interface Sci. 15 (2010) 435–444.
[254] L. Nyström, M. Malmsten, Surface-bound microgels –from physicochemical properties to biomedical applications, Adv. Colloid Interface Sci. 238 (2016) 88–104.
[255] J.P. Silva, C. Goncalves, C. Costa, et al., Delivery of LLKKK18 loaded into self-assembling hyaluronic acid nanogel for tuberculosis treatment, J. Controlled Rel. 235 (2016) 112–124.
[256] N.Y. Chew, H.K. Chan, The role of particle properties in pharmaceutical powder inhalation formulations, J. Aerosol Med. 15 (2002) 325–330.
[257] S. Belotti, A. Rossi, P. Colombo, et al., Spray dried amikacin powder for inhalation in cystic fibrosis patients: a quality by design approach for product construction, Int. J. Pharm. 471 (2014) 507–515.
[258] A. Schuster, C. Haliburn, G. Doring, et al., Safety, efficacy and convenience of colistimethate sodium dry powder for inhalation (Colobreathe DPI) in patients with cystic fibrosis: a randomised study, Thorax 68 (2013) 344–350.
[259] P.C.L. Kwok, A. Grabarek, M.Y.T. Chow, et al., Inhalable spray-dried formulation of D-LAK antimicrobial peptides targeting tuberculosis, Int. J. Pharm. 491 (2015) 367–374.