

**NOVEL ANTI-MICROBIAL
PEPTIDOMIMETIC COMPOUNDS AND
METHODS TO CALCULATE
ANTI-MICROBIAL ACTIVITY**

[0001] This application claims priority from U.S. provisional application 60/876,377, filed Dec. 21, 2006, the entire contents of which are incorporated herein by reference.

FIELD OF THE INVENTION

[0002] The focus of this application is novel membrane disruptive antimicrobial peptides (AMPs) with increased selectivity and potency against specific bacterial strains. As described below, this invention encompasses synthetic antimicrobial peptide analogs having certain formulas, including the un-natural hydrophobic amino acids tetrahydroisoquinolinecarboxylic acid (Tic) and octahydroindolecarboxylic acid (Oic). These antimicrobial peptides (AMPs) are useful to treat infection in humans and other mammals caused by such bacteria as Gram positive bacteria, Gram negative bacteria and Mycobacteria. Many of the AMPs also exhibit the property of reduced hemolytic activity.

BACKGROUND OF THE INVENTION

[0003] Antimicrobial peptides (AMP) have evolved in almost every class of living organism as a defense mechanism against invading micro-organisms including bacteria, fungi, protozoa and parasites.^{1, 2} As of 2004,³ over 800 antimicrobial peptides had been isolated and characterized from various organisms including humans,⁴ amphibians,⁵ insects, mammals, birds, fish and plants.² AMPs are generally small (10-50 amino acid residues) highly positively charged (+3 to +9)⁶ amphipathic molecules with well defined hydrophobic and hydrophilic regions.^{3, 7}

[0004] The exact mechanism of membrane-induced cytotoxicity of these peptides is currently a topic of debate in the literature.⁸ AMPs are broadly divided into two major classes 1) membrane disruptive and 2) non-membrane disruptive. [Powers, 2003 #3]⁸ All membrane-disruptors follow specific steps in the process of interacting with their target cells.⁹ The first step is the attraction (or movement of the AMP through bulk solution to an area near the surface) of the AMP to the surface of the membrane.¹⁰ The driving force for this attraction is the electrostatic interaction between the positively charged basic amino acids on the AMP and the negatively charged acidic phospholipids found in the targets cell's membrane.^{11, 12 13} The second step is binding of the AMP to the surface of the membrane.^{10, 14} In this step the AMP attaches to the surface of the membrane by locating the positively charged side chains relatively close to the negatively charged polar head groups of the phospholipids followed by insertion of the hydrophobic side chains of the AMP into the hydrophobic core of the membrane. During this process conformational changes occur on the AMP that stabilizes the attractive electrostatic and hydrophobic interactions while concurrently minimizing the repulsive interactions between the AMP and the membrane. At lower concentrations the long axis of the AMP is oriented parallel to the surface of the membrane, and is called the S-state.¹⁵ As the concentration of the AMPs increases on the surface of the membrane a critical concentration is reached where aggregation occurs forming complexes of 4-6 AMPs. This induces a change in the orien-

tation of the long axis of the AMPs from parallel to perpendicular relative to the membrane surface resulting in the insertion of the aggregate AMPs in to the membrane forming a transmembrane pore, and this is called the I-state.^{14, 10}

[0005] The membrane-disruptors can be further divided into two sub-classifications; 1) cell selective (i.e. magainins and cecropins), 2) non-selective (i.e. melittin and pardaxin).¹⁶ As the name implies, cell selective AMPs exhibit potent activity against bacterial cells while being inactive against mammalian cells. Non-selective AMPs are active against both bacterial and mammalian cells. The selectivity of AMPs for bacterial vs. mammalian cells is believed to be based on the differences in the chemical composition of the two cell membranes.^{2, 3} Bacterial cells contain a high percentage of negatively charged phospholipids while mammalian cells contain a much higher concentration of zwitterionic phospholipids.¹² Other differences also exist between the two including; membrane composition (sterols, lipopolysaccharide, peptidoglycan etc.)¹, structure, transmembrane potential, and membrane polarizability. In addition to the differences between eukaryotic and prokaryotic cells the membranes surrounding different types of bacterial cells are also different. The lipid bilayer of Gram positive bacteria is covered by a porous layer of peptidoglycan, while the structure of Gram negative bacteria is more complex consisting of two lipid membranes containing lipopolysaccharides and porins.^{17 2} The outer membrane of mycobacterium is the most complex of the three, consisting of an additional very thick mycolate-rich outer coat which is very difficult to penetrate.

[0006] It is generally no longer accepted that AMPs are uniform and indiscriminant membrane detergents. As more information becomes available regarding the sophisticated and thematic structure-activity relationships underlying distinct mechanisms of action among AMPs, there will be a greater understanding of their likely multiple roles in antimicrobial host defense.¹ There is growing evidence in the literature that the selectivity and potency of a specific AMP is determined in a large measure by the chemical composition of the target membrane.⁸ Thus, it is reasonable to postulate that the membrane's physicochemical surface interactions with the physicochemical surface of the AMP defines the organism specific potency and selectivity.^{2, 8, 17, 18} As pointed out by Toke³, understanding how the physicochemical interactions between AMPs and the lipid composition of their target cells are important that they define organism potency and selectivity which is critical for the development of AMP derived drugs.

SUMMARY OF THE INVENTION

[0007] The evolution of drug resistant bacterial strains is one of the most critical problems facing modern medicine and requires the development of drugs that exhibit anti-bacterial activity via novel mechanism. Antimicrobial peptides (AMPs) interact differently with different bacterial strains (such as Gram positive, Gram negative and *Mycobacterium*) based on the differing chemical composition of their respective cell membranes. The inventors have discovered that small changes in the structure and physicochemical properties of the constituent amino acid residues can lead to major changes in the potency and selectivity of a particular AMP for a particular bacterial strain. Therefore, by selection and placement of natural and/or un-natural amino acid residues with specific physicochemical properties, the inventors have