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Review of Literature

Medical & Clinical Research

Antimicrobial peptide surrogates based on active moiety of dermasetin s4: A history in Peptide-mimetics Research

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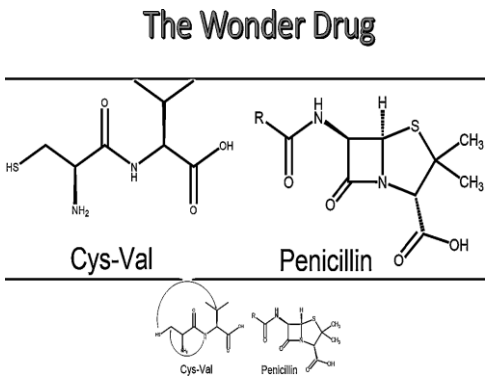


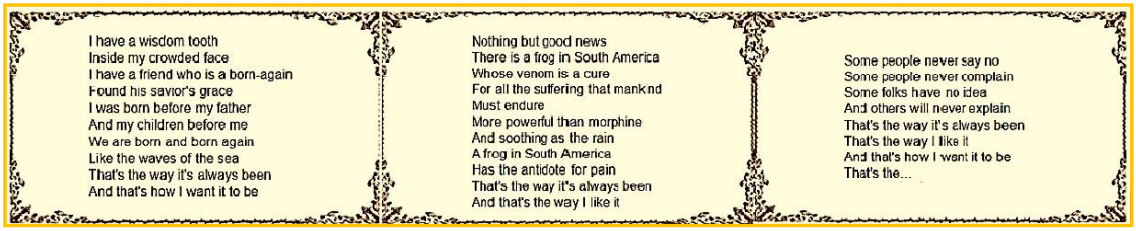
A personal Remark

I am occupied with this problem for over 20 years now, I hope the remedy is found for all that are victims of the harmful microbes, but the problem is really huge and worldwide. I am an example that in 2012 survived blood-sepsis but was treated.as a last resort, with veterinary medicine used for pigs and chicken, COLISTIN (POLYMYXIN B). Waving for 1 week from worlds (saw the Whalefish and the wild Oxen) to this world (BIBI, SURE). As you see, it was useful and practical, but triggered renal problems, as expected. Now I am under the surveillance of many physicians in Beilinson, but they keep me alive. I know what this is, believe me. What I can do is some research.

Foreword

It is the "septic shock" defined as sepsis with the blood pressure that requires Blood vessel care agents to maintain average blood Pressure of more than 65 mmHg and serum lactic acid level exceeds 2 mmol/L (18 mg/dL) after adequate resuscitation of fluids. Sepsis has a risk of death and long-term morbidity. Septicemia is dangerous blood Pollution known as blood poisoning. This happens when the bacterial Infection in other parts of the body, enter the bloodstream. The danger because of the bacteria and they're secreted in their effluents produced toxins [1,2]. A used diagnosis can quickly identify pathogens in the patient's intestines Microbials, researchers have found evidence that the patient's own Microbiome may be the source of blood purchased at the hospital Infections (BSI), Sepsis life-threatening [3,4].

Here is a frog in South American jungles….this is the beautiful song by Paul Simon Señorita With a Necklace of Tears, Paul Simon, the second verse [5]:



So we found the green little frog, Phyllomedusa Savage [6], from the Amazon/Orinoco jungles, whose skin contains many antimicrobial peptides, and focused on Dermaseptin S4 [7,8] as one of the most active one, and this in many ways.

Antibacterial Agents

Abstract

Common failure and acute use of antibiotic therapy is a severe public health problem. The most obvious reason is that the successful use of any therapeutic agent is impaired by the development of bacterial resistance. An example is antibiotics of aminoglycoside, such as gentamicin and kanamycin, directly to the ribosome target,

but the mechanisms in which these drugs cause cell disruption are not fully understood. The era of antibiotics founded on intracellular target components seems to end. Also the period of antibiotics that interfere with cell wall synthesis loses momentum. The long list of antibiotics for its families is becoming inadequate as it barely provides for common non-social or other acquired bacterial infections. It seems that the utilization of antimicrobial peptides (AMP, samples include cecropins, from insects, magainins from amphibians and cathelicidins, from mammals) and their way to eliminate bacteria by cell wall disturbance is one of the few clues to the problem of microbial resistance, the elimination of inhibitory cells, dormant and mutant cells may become a real possibility and bring cure to those suffering from the incurable infection caused by the killer bacteria.

Antimicrobial peptides are a weapon of nature to combat the invasion of bacteria as host defense agents, the first line of defense. However, natural peptides suffer from serious shortcomings first on their list being peptides susceptible to enzymatic digestion. The approach of peptidomimetics [9] bears many advantages over the gift of nature and that is:

Stability in the enzymatic world.

- ✓ The option of human design.
- ✓ Use of non-natural components for building mimicking architecture.

An approach based on these lines enables the introduction of the innovative design of components that may assist in destruction but which are new to nature. This may allow the exploration of the path of selective elimination of bacteria in the presence of beneficial vegetation.

There are many hurdles in this way: it is necessary to learn about the mechanisms in which AMP disrupt the cell membrane in full detail. Today, there are many ways in which the membranes are disassembled. It was found that low molecular weight (MW=500) mimics non-peptides are capable of eliminating bacteria and small structural change may lead to preference in elimination according to the desire of positive bacteria or Gram-negative bacteria. The cruel choice of resistant bacteria, coupled with inadequate investment in antibacterial research, has led to a steady decline in the efficiency of existing treatments and a minority of innovative structural classes that will replace them or supplement their use [10].

In this publication we review the situation in antibacterial agents with emphasis on the new tendency in developments based on antimicrobial peptides and their hosts. The current situation which harms us with nosocomial infections, may bring us reminiscent times in the picture: Selman Waksman and his assistants [11,12].

The golden age of antibiotic discovery began with systematic testing of soil microbes by Selman Waksman (pictured centre).

Recover the lost art of drug discovery

Opening Words

Antimicrobial agents are part of the world's most widely used drug treatment and are often used. A fundamental philosophy of medical treatments, including the combination of antibiotics, is that different drugs work through different mechanisms, and that the consequences of using multiple drugs will be somewhat synergistic. Because they have different adverse effects on dosage restriction, they can be given together in full doses in the regimens.

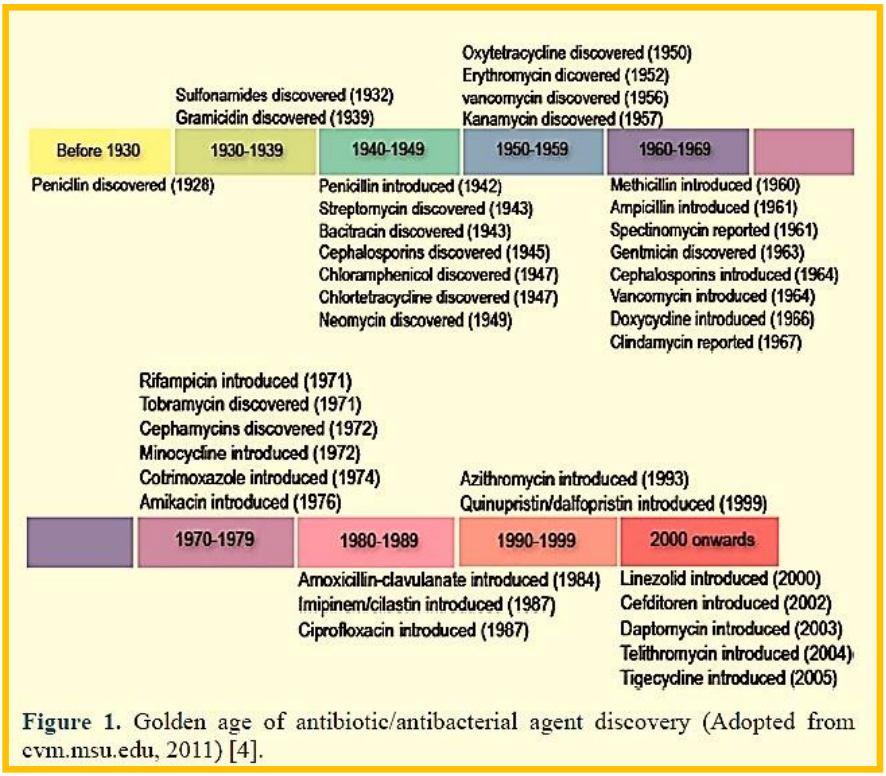
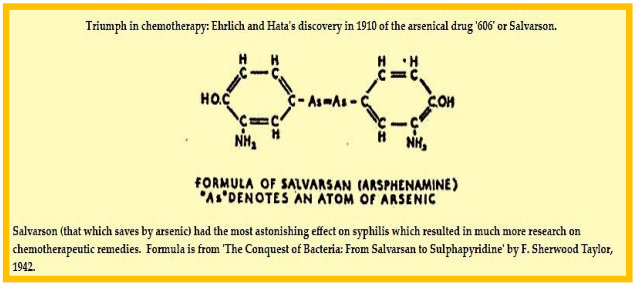


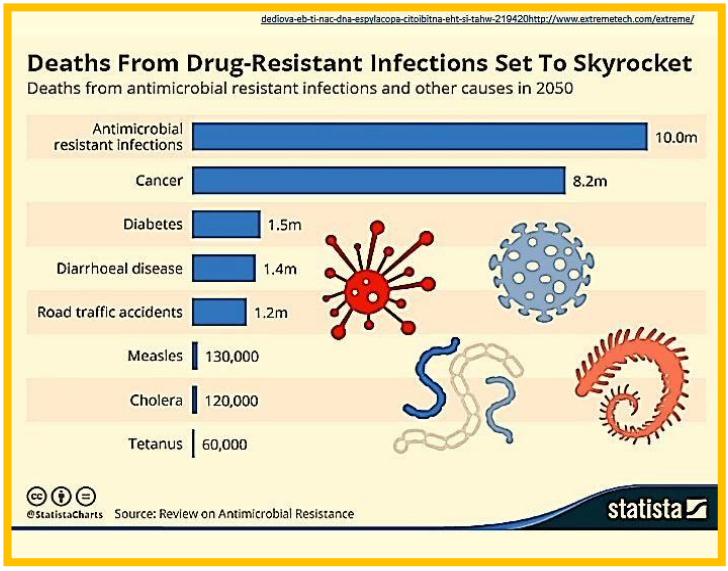
Figure 1. Golden age of antibiotic/antibacterial agent discovery (Adopted from cvm.msu.edu, 2011) [4].

This idea prevailed through the years of infection therapy. In the last 80 years since the first treatment of syphilis with salvarsan by Ehrlich. The initially expressed idea of looking for the "magic bullet"[13] as an "ideal" agent that will cure infections is pursued until now, but the bullet is still elusive. Ehrlich concentrates on work to create his "magic bullets"-chemicals injected into the blood to fight various diseases, thus pioneering antibiotic therapy for infectious diseases (later adopted by others to fight cancer).

Ehrlich and his co-workers [14] tried hundreds of chemicals on the microbes that caused syphilis. In 1909, Ehrlich's new colleague Sahachiro Hata (1873-1938) brought with him a method of producing syphilis infections in laboratory rabbits, and discovered (1910) that drug no. 606 worked. The first 'magic bullet' had been found, and was marketed under the name Salvarsan. This was the first "magic bullet".

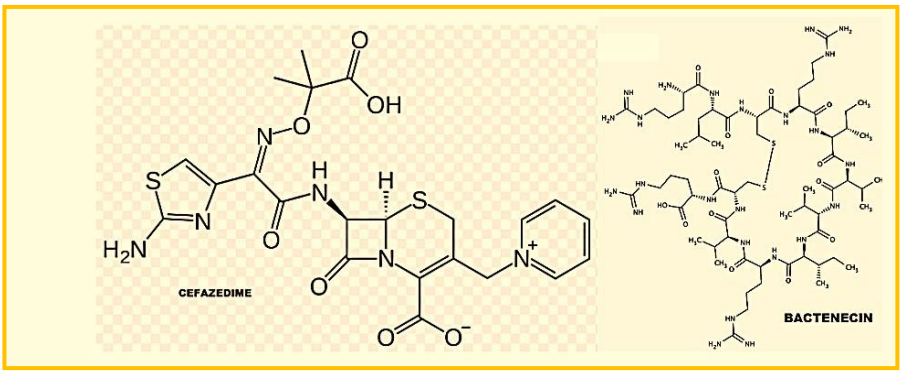


In the last period, of eight decades, with a peak at the period of the years 1960-1980, Humankind enjoyed on the basis of e4hrlich's findings, a period of triumph over the ancient dwellers. Our nemesis, the most ancient dwellers of this planet: the microbes (archaea, bacteria, fungi, viruses and more). Since the days of Ehrlich, the progressive development of technology, in chemistry and Biotechnology, provided agents of different nature: those from chemical synthesis (Sulfa drugs, Domagk [15]) and others from bio-synthesis (penicillin, Fleming [16]). Materials that could eradicate the broad spectrum of microbes very efficiently and allowed in this short era the extension on human life longevity from 40 years on average to 80 years of today [17]. People took it for granted, these were days people thought they would never end, not knowing that the "sudden bullet bullets" did not completely eliminate the bacteria, the few organisms left on the margins spread, but this time to unknown new races that were resistant to "agents wonder". And here we are again today, in front of the world of germs without weapons and active at this stage of the campaign [18]. One of the significant mistakes exercised by the healthcare system was and is still the abuse, over application of the antibiotic drugs. The common and use practically without a barrier is one of the main reasons why humankind is now in the grim situation of "fighting without effective weapons" This is today one of the leading killers of people and will no doubt skyrocket soon [19].



The nosocomial microbial infections are currently on their way to become the considerable morbidity causing factor in our life. One of every 25 [20] entering a healthcare facility is infected, millions die due to lack of remedy, a novel idea or strategy is badly needed. The antimicrobial peptide may bring remedy in cases of bulky disease. For instance [21], Burkholderia pseudomallei is a B type agent causing Melioidosis, a chronic acute disease with septicemia.

The current treatment regimen is a heavy dose of antibiotics such as ceftazidime (CAZ); However, the risk of recurrence is possible. Activity bacteria against B. pseudomallei and examine the membrane disrupting capabilities of powerful antimicrobial peptides: bactenecin. All peptides showed an activity of 97% in bacterial activity at 20 mm, with slightly higher bactenecin activity.



Peptide antibiotics are an alternative to traditional antibiotics as they exhibit rapid action and are less tending to result in the development of resistance.

In view of the discouraging current states of affairs and the alarming perspectives (see chart above), the work is spreading in mainly two different strategic option:

1. “Let them become sick, we will cure the disease”-Pharma industry. The pipeline contains:

a) Improving existing drugs.

b) a better knowledge of the mechanism of action needed for

2. The design an synthesis of DE-NUVO antimicrobial agents.

c) Isolation of active agents from the vast pool of natural products

d) Design and synthesis of surrogates of “natural” bioactive compounds. The design and synthesis of surrogates of bioactive compounds.

As things developed, more complex molecules were introduced as drugs (examples above), and the microbes developed resistance to the drags.

2. Prevention of infection “keeping the microbe-free environment in healthcare facilities”- more effective sterilization and antiseptic treatment of the whole facility-The Chemical industry.

a) There are better disinfecting agents.

b) Better hygiene behaviors.

c) Novel sterilization agents are needed to keep the whole installation sterile.

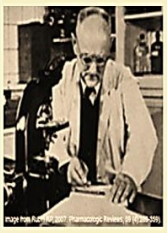
A combination of the two attitudes is penetrating, slowly, the healthcare system. Yet, both have the severe drawback that enables the proliferation of the “Nosocomial infections” death pandemic.

The Golden Age of Antibacterials

In 1928, **Fleming**'s major medical breakthrough came about as he serendipitously discovered penicillin, later to be claimed as the miracle drug of the 20th century. However, the impact of this discovery was not realized until the 1940s, when its applicability as a therapeutic agent was made possible by **Florey and Chain**. Blamed for this delay was the lack of biochemical and microbiological expertise at that time, as well as the lack of interest and support from the scientific community brought about by previous experience with the failure of pyocyanase and the toxicity of Salvarsan.

In 1935, a breakthrough that ushered the era of antibacterials was made by the German biochemist **Gerhard Domagk** at the Bayer Laboratories of the I.G. Farben company in Germany[19]. He discovered and developed the first **sulfonamide**, a synthetic red dye more popularly known by its trade name of Prontosil, the first commercially available antibacterial. Impressive clinical successes resulted in a sharp decline in mortality due to killer diseases such as meningitis, child bed fever and pneumonia. Domagk's discovery saved many lives, including prominent figures such as Winston Churchill[6] and Franklin D. Roosevelt, Jr., son of then US President Roosevelt.

Inspired by the groundbreaking work of Domagk (with sulfa) and Fleming, Florey and Chain (with Penicillin), a number of subsequent antimicrobial discoveries quickly followed. To this day, newer antimicrobial compounds continue to be discovered and introduced, although the rate has slowed considerably.



Domagk thought that because dyes have affinity for bacterial cells, they may possibly alter their growth once taken inside. He tested the synthetic newly patented red dye, Prontosil. Luckily, he also tested this chemical in mice; if he relied on test tubes alone, no activity would have been observed and the discovery would have not been made. This is because the active component of the dye, the sulfonamide requires release during a necessary metabolic processes *in vivo*[16].

The first to receive this was drug his own young daughter. A needle was accidentally embedded in her wrist bone, eye first, and broke off when she fell from the stairs. It was surgically removed but a secondary streptococci infection, which Domagk himself identified, resulted in a life-threatening fever. Left with very little option, he asked permission from the attending doctor to give her Prontosil. His daughter recovered almost immediately[20].

For this monumental discovery, Domagk was awarded the 1939 Nobel Prize in Physiology or Medicine, but, being a German national, was forbidden by the Nazi regime to receive it. He was instead arrested and jailed. He finally received the award in 1947, after the war.

One morning in September 1928, Sir Alexander Fleming, who had just returned from vacation, returned to a laboratory full of filthy, staphylococcus. While disinfecting old plates, he noticed how much of a delay in bacterial growth around the mold infection was interested in because his previous work was on finding active antibacterial agents. He later identified the penicillium sphere, called the penicillin extract, and published his findings in the British Journal of Experimental Pathology in 1929. After realizing the difficulty of cultivating the template and purifying the active agent, he thought this discovery had little application.

However, about nine years later, pharmacist Howard Fleury and biochemist Ernest Boris Chain read his work and are interested in this research even more chemically.

Then they successfully purified and developed a large-scale antibiotic after that saving millions of lives since its introduction in 1942, was later praised as a miracle medicine of the 20th century [22].

On the grounds of his extraordinary contribution to science, Fleming, Florey and Chain shared the Nobel Prize for Physiology and Medicine in 1945.

Seeking Bioactive Strain Selective Antimicrobial Agents

The technology [23] described here is based on the defense mechanisms of nature. The membranes of microbial cells are destroyed. This unique technology is a new type of microbial treatment that activates the living microbes themselves and processes for its elimination. Based on natural products, anti-microbial peptides that are the basis for innate immune systems of all living organisms on the planet. These compounds were considered a potential treatment due to their activity in the broad spectrum and their proven ability to prevent antimicrobial resistance, but their clinical and commercial developments have certain limitations, such as sensitivity proteases and high cost of peptide production. To overcome these problems, many researchers have tried to develop short active peptides, and their imitations vary with better properties while maintaining the basic properties of natural AMPs such as cationic charge and an amphitheater structure. Biotic motifs which are identified sequences of natural AMPs may be used. The backbone is synthetic substitutes of these peptides. It was determined that in many cases only small sequences of AMP are bio-active, it can be used as a backbone for the design of synthetic imitations of antimicrobial peptides (SMAMPs) with Excellent features.

The ability of microbes to develop resistance against an antimicrobial agent requires searching, planning, and preparing a medication to combat a severe and life- threatening problem. The (WHO, World Health Organization) recently published a report focusing on antimicrobial resistance and global monitoring with the request for new drugs and healthcare services for the world's population. In the last century, the antibiotic revolution has contributed to the elimination of many infectious diseases (cholera, syphilis, pneumonia, Tuberculosis, for example) to dramatically increase human life, while also contributing to the treatment of World War II injuries from lethal infections, while building microorganism resistance has always been a determining factor in seeking better and more effective agents for the elimination of bacteria.

Antibacterial peptides have multiple roles in protecting the vaccine [24]. The case with Gramicidin (GS) may oppose the example. Although the mechanism of action of the GS is not fully understood, it is common that the peptide kills bacterial cells through stable stability and permeabilization of their cytoplasmic membranes. Synge [25] In the early years of the region, we examined the cyclic peptide Gramicidin-S [26,27], currently applied only to local antibacterial treatment. Katchalski (Katzir) prepared poly-lysine and investigated the quality of their biocides [28,29] as bacterial killers. It should be noted that these peptide peptides are short [30] and do not result in any development of resistance to E. coli measurement. MIC (minimal inhibitory concentration) [31,32] Antibacterial peptides have multiple roles in immune defense . The case with Gramicidin (GS) may contradict this. Although the mechanism of action of the GS is not fully understood, it is common that the peptide kills bacterial cells through stable stability and permeabilization of their cytoplasmic membranes. However, this perception of the bioactivity of amphipathic AMPs and probably their substitutes may be too approximate, although cationic amps have varied secondary structures, their surfaces are uniformly amphibious with two hydrophilic hydrophobic residues. The microstructural architecture and microbial factors of microbial peptides (SMAMP) that mimic peptides for host protection include charge, amplifiability, hydrophobicity, elasticity and capacity (33) are key factors considered. The Lys cylinder in Cecropin-Mellitin is prominent: Feix reported that peptide- enriched peptides maintain vigorous microbial activity and, most importantly, significantly reduce toxicity toward eukaryotic cells. Although peptide sequences are very diverse, most host protection peptides appear to act by direct lysis of the pathogenic cell membrane. They are defined as active molecules by Takahashi, Pikes and Sato [34]. While their lytic activity is usually not mediated by a chiral center, [35] the exact mechanism behind this activity is not entirely understood [36,37].

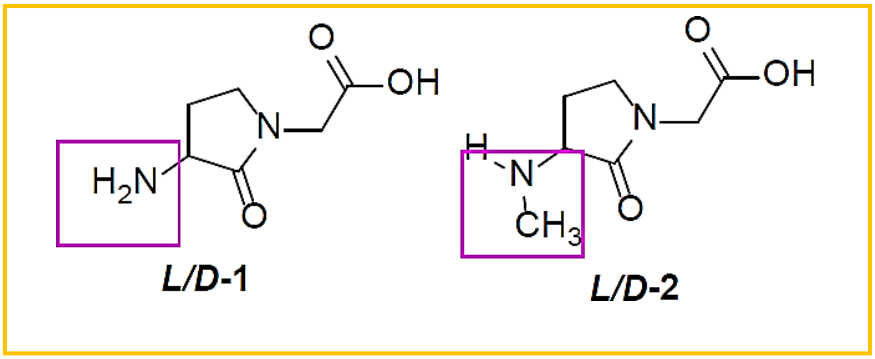
Harnessing the outer receptor of the cell wall for the better activity of SMAMPs is attractive, especially to combat Gram-negative bacteria. Swendsen and his associates reported that SMAMP combines serum albumin. Ting-Wei Chang reported the outer membrane Lipoprotein Lpp in gram-negative bacterial cell surface receptor for antimicrobial cation peptides. Since the architecture of receptors such receptors are abundant in β -turn moieties, imparting β-turn emulators [38,39,40] may strengthen such interactions with the outer membrane.

There are pressing and urgent demands for new effective antibiotics that are effective against drug-resistant bacteria without contributing to the development of resistance [41,42]. Antimicrobial peptides (AMPs) are promising romantic antibiotics because they exhibit a broad microbial spectrum and are not easily objectionable.

For clinical applications, it is essential to develop robust AMPs with less toxicity against host cells. We have designed a short cationic peptide epic consisting of two functional domains (KAAAK) embedded in the surrogate peptide composition.

Because of their mechanism of action [43], antimicrobial peptides (AMPs) showed little bacterial, fungal and viral resistance [44]. We designed and developed short peptides containing β rotation [45] mimicking as “shelter” [46] “mode and two lysines indexed [47] terms in their sequences and with amphibious structures based on imitation cation [48] of natural antimicrobial peptides occurring at concentrations Deficient, less than 10 μm. These short peptide peptides exhibit this intense antimicrobial activity against a wide range of bacteria including E. coli and methicillin-resistant Staphylococcus aureus without the acute hemolytic activity of the agglutination of erythrocytes [49]. It should be noted that these short peptide substitutes did not lead to the development of resistance to E. coli measurement.

MIC (minimal inhibitory concentrations) [50] Experiments indicate that β-turn- Freidinger D and L are based on nearly identical anti-microbial catalytic peptide



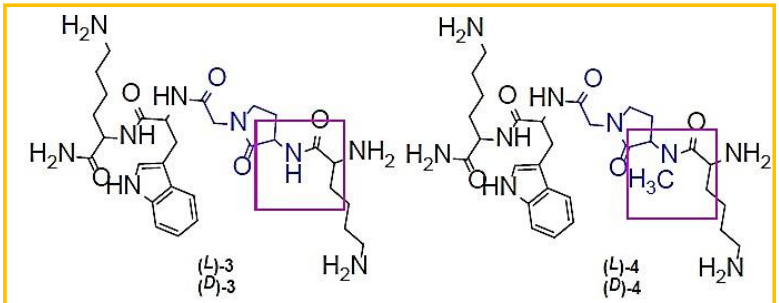


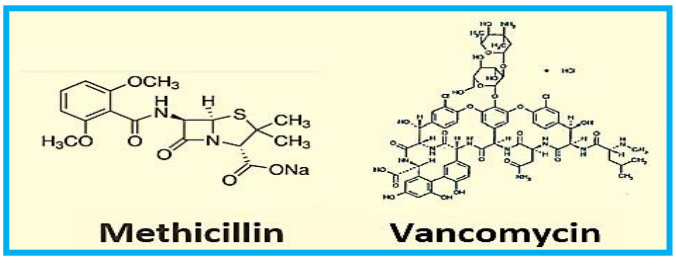
Fig. 2. Four peptidomimetic surrogates that were used in this study.

(CAMP) in Gram and Gram bacteria testing [51]. These results indicate similar behavior of "artificial" [D] samples and "natural" [L]-peptide when bacterial membrane binding has, however, chiral sensitivity in human red blood cells (RBC) hemolysis [52] and therefore a window of opportunity to reduce toxicity) By selecting the appropriate chirality of the surrogate peptide. The peptide surrogate design principle offers significant flexibility and diversity in the creation of new antimicrobial agents and possible biomedical applications. The potential of short-term β-turn AMPs for careful studies to avoid the elimination of "friendly" microorganisms is discussed based on proteins and external membrane bacteria [53]. Our studies may have contributed to further understanding of how CAMPs (Cationic Antimicrobial Peptides) feel the microbial membrane [54] and provide a new direction for the development of new membrane disruptors [55,56]. A temporary structure has been identified which can connect to external membrane proteins of harmful Gram (-) bacteria [57], and modifies the biological activity of antimicrobial peptide mimics [58-60]. The different architectures of the cell wall [61] of Gram- positive bacteria and gram-negative bacteria may present a tool for careful selection of targets for elimination by microbial and host peptides. B and N methylation may present a toolbox for designers and synthetic people to shape and synthesize the necessary impulse to rapidly emerging bacterial epidemics, the antiviral agents of romance. Unfortunately, GS displays local applications.

Perspectives: Antimicrobial Peptides Surrogates

Introduction

The Fast Development of a microbial nosocomial pandemic that is in many cases estimated today to cause a dramatic increase in the morbidity of people that contracted microbial infections caused mainly by bacteria are part of the innate immune response found among all classes of living beings. The Antimicrobial peptides (AMP) [62] are also called host defense peptides of which 2000 different short (5-50 amino acids sequences) such polypeptides were isolated and identified. The current situation with the antibiotic drugs is that bacteria became resistant to all medicinally applied drugs, this includes Gram-positive bacteria that are classified as of instance Methicillin-resistant *Staphylococcus aureus*, (MRSA) and the Gram-negative that are in the group of Carbapenem-resistant Enterobacteriaceae (CRE).

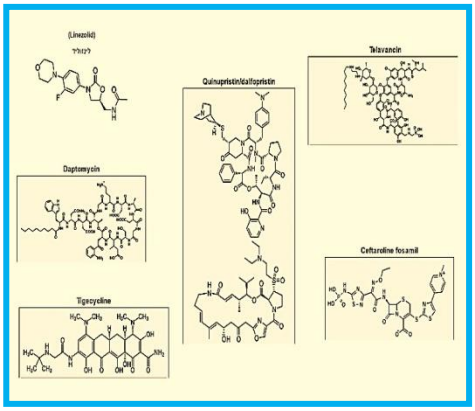


Bacteria with positive bacteria are a common cause of blood flow and other infections in patients hospitalized in the United States, for example and the percentage of infections in the blood in neuro somal surgeries caused by bacteria with positive antibiotic bacterium increases. In the United States, about 60% of staphylococcal infections in the intensive care unit are now caused by MRSA, and percentages continue to rise.MRSA-induced MRSA outbreaks are usually the result of the spread of MRSA, which is transmitted from patient to patient.

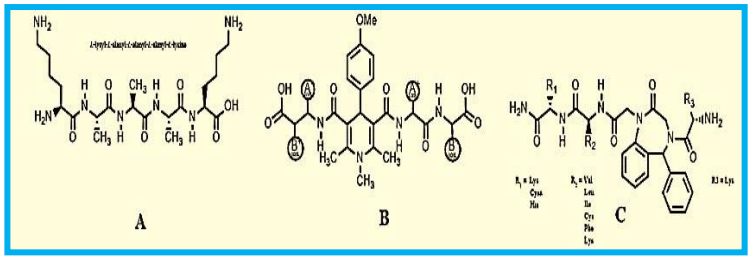
Over the past ten years, the medical community has seen the development of several antibiotics active against resistant gram-positive organisms, including linezolid, daptomycin, tigecycline, quinupristin-dalfopristin, telavancin, and ceftaroline. These agents have expanded treatment options but also have their limitations. Resistance to each one has been observed in individuals not previously exposed to antibiotics. Future study on the efficacy of these agents and careful monitoring of the molecular epidemiology of the resistance mechanisms is of high priority.

The state of multidrug-resistant Gram-positive organisms is now being overshadowed by the emergence of resistant gram-negative pathogens. In contrast to Gram-positive organisms, the pharmaceutical pipeline for antibiotics active against multiple

drug resistance (MDR) Gram-negative organisms is dry with no new medications in advanced stages of clinical development. Due to this emergence of highly resistant pathogens, clinicians have been forced to use antibiotics with known significant toxicities and poorly studied benefits.



Some Drug Molecules for Gram-Positive Infections



In 1948 Synge [63] reported on the Synthesis of Some Dipeptides Related to the anti- microbial peptide (AMP) Gramicidin S., These dipeptides were required for the study of the structure of Gramicidin S. in the frame of his efforts to learn about the very high antibacterial activity of the cyclic peptide.

Currently, AMPs represent one of the most promising future strategies to combat drug-resistant infections and bacteria due to their mechanism of bacteria eradication by cell wall disruption. It is evident by the increasing number of studies to which these peptides investigated. It is apparent: our need for new antimicrobials becomes more pressing due to nosocomial infections and incompetence of currently applied agent to combat resistant microbes on the one hand. On the other hand neurodegenerative diseases are believed to start in inflammation and infection which might require [1b] treatment with modified AMPs.

The problem remains: can we develop novel drugs based on the design principles of first molecules?

The advantage of the AMP is that most of them act in the broad eradication spectrum of bacteria in a new mechanism that is less likely to allow the development of resistant strands of the microbes. However, today many mechanisms [64], have many drawbacks. Difficulties identified when AMPs. Those compounds considered as potential therapeutic agents. The currently applied Antimicrobial peptide (AMP) based drug-like compounds suffer from one major drawback that could jeopardize efforts for therapeutically using: they are not "strain selectively" [65], kill the microbiome "good" and the "bad" bacteria in a similar way [66]. Eradication of all bacteria takes place is a similar efficiency [67]. [68]. To overcome the drawbacks, and introduce features that are not present in the AMPs themselves. Some of the famous AMPs is isolated from the skin of an African todd. Magazines. The company that was established with the same name realized the antimicrobial therapeutic potential in mimics of the natural Magazine, namely a semisynthetic polypeptide surrogate Paxiganan [69] as an analog of Magazine 2. Surrogate agents that are Mimics of AMPs are collectively called peptidomimetics. The types of modifications that are introduced are generally modeled after the structural requirements which are known to influence AMP activity. Attempts to conserve features like positive charge and amphipathicity are made to ensure the antibacterial activity of the mimetic compounds. The mimics, often constructed with a different backbone (i.e., not based on -amino acids) or may carry dislocated side chains to overcome the low bioavailability and lack of metabolic stability found for traditional AMPs [70].

We pursued the quest for the preferential eradication of microbes. We focused our interest on our finding that an antibacterial motif, Lys-Ala-Ala-Ala-Lys A, identified in Dermaseptin S4 could be a subject for mimicking since its surrogates show similar broadband [71] antimicrobial activity B and C [72]. In particular, compound C where the β-turn mimics benzodiazepine is incorporated into the hydrophobic part of the molecule.

Usually, the naturally operating in the innate immune system is composed of many amino acids in a particular sequence. Only a

short sequence was found to possess most of the antimicrobial potential. A general presentation of a typical process towards a Surrogate for the naturally active polypeptide [73] is as follows:

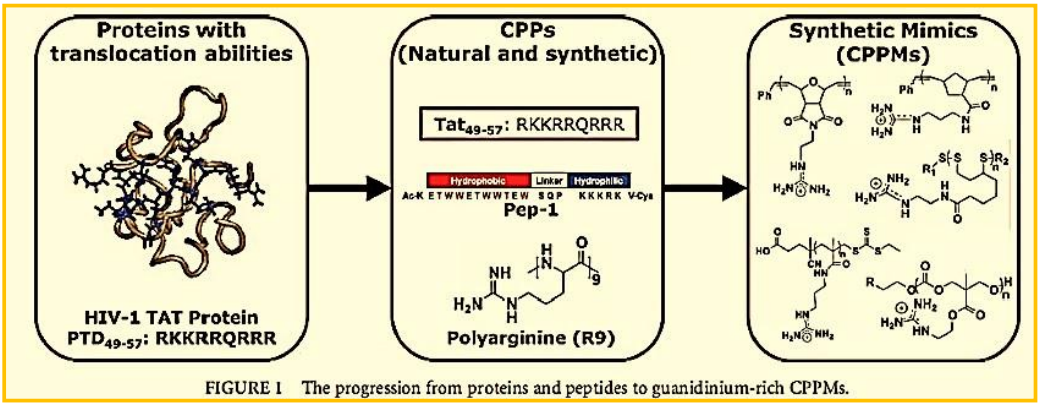
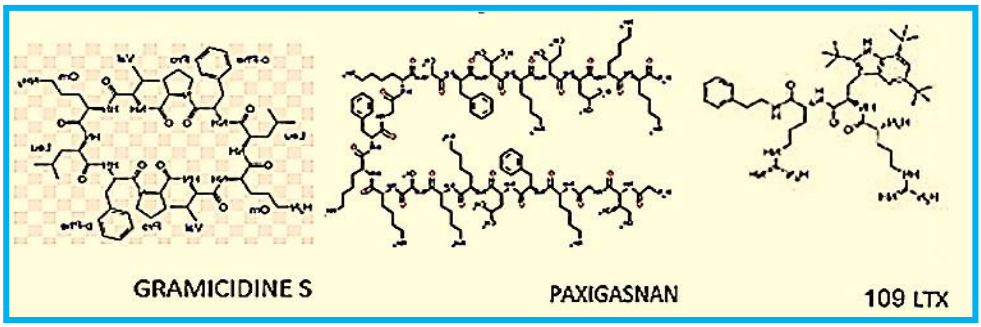


FIGURE 1 The progression from proteins and peptides to guanidinium-rich CPPMs.

The strains of harmful bacteria become more resistant to drugs, but also live in the same organism, environment as another beneficial and vital pet of microorganisms that exist in the human intestines, "useful" different strands of raids, Bacteroidetes, Actinobacteria, and Proteobacteria. We want to selectively eliminate the "bad" microorganisms and leave the "useful" intact. Currently, one of the highest obstacles in the application race is the selectivity of eliminating bacteria. Mitsuki and others investigated the possibility of cell selectivity [74]. The presentation of an AMP with the membrane cannot be explained only by a particular sequence of amino-acid pattern or motif; instead, they originate from a blend of physicochemical and structural features [75] including residue composition, size, overall charge, secondary structure, hydrophobicity and amphiphilic character [76].

Today, mimicking the antimicrobial activity of AMPs has entered the stage of the application as topical drugs, this due to the nonselective eradication of microbes. In this respect, application of GramicidinS was followed by Paxiganan and the newly manufactured LTX 109, are practically a very broad spectrum microbe's eradication agent with limited medicinal value due to lack of selectivity. They kill them all Peptide surrogates the early years.

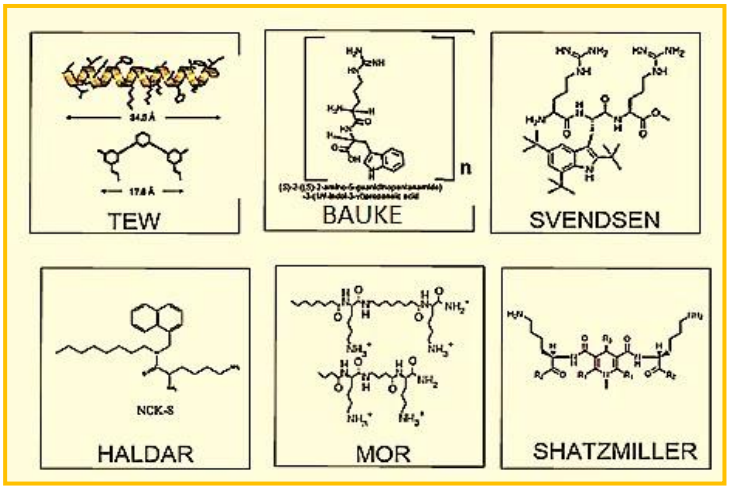


It is believed that cyclic form of AMPs contributes to their extraordinary high eradication ability [77] by a hydrogen-bonded tubular architecture of the self- assembled, eight-residue cyclic D, L-a-peptide, and modes of membrane permeation accessible to peptide nanotubes.

The current situation focuses on a few research efforts that handle small molecules with a molecular weight around 400-500 and i8n respecting the rules of Lipinski rule of 5 [78].

Surrogates of Short Peptide Sequences for Drug-Like Agents

The work in this frame was to identify short bioactive (antimicrobial) peptide sequences and in AMP from various sources (Human, Amphibians, Fish, insects, Serial, etc. In the following drawing are represented a few structures of surrogate antimicrobial structure that was designed by natural motifs found in AMPs.



Peptide Surrogates-the Second Pulse

Tew [79] and his colleagues designed the tricyclic aromatic structure by the general idea of the amphipathic nature of AMP, in particular, the work of Vogel [80]. Park [81] and Bauke [82] explored the motifs WK and WR respectively as a unit for the eradication of bacteria. Svendsen and partners established a corporation for the development of antibacterial medicins based on Lactoferricin turn [83,84]. On this basis the Topical drug molecule LTX 109 was developed by Svendsen and the people from LYTX [85]. Haldar and his group investigated the use of [86] Aryl-Alkyl-Lysines as Agents That Kill Planktonic Cells, Persister Cells, Biofilms of MRSA and Protect Mice from Skin- Infection. Mor and his coworkers applied a lysine amphipathic unit to construct antimicrobial polymers [87].

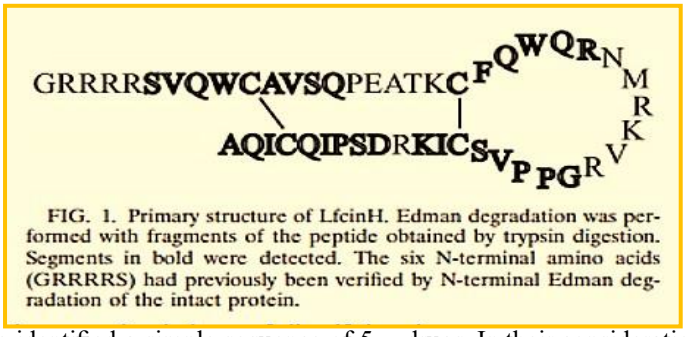


FIG. 1. Primary structure of LfcinH. Edman degradation was performed with fragments of the peptide obtained by trypsin digestion. Segments in bold were detected. The six N-terminal amino acids (GRRRRS) had previously been verified by N-terminal Edman degradation of the intact protein.

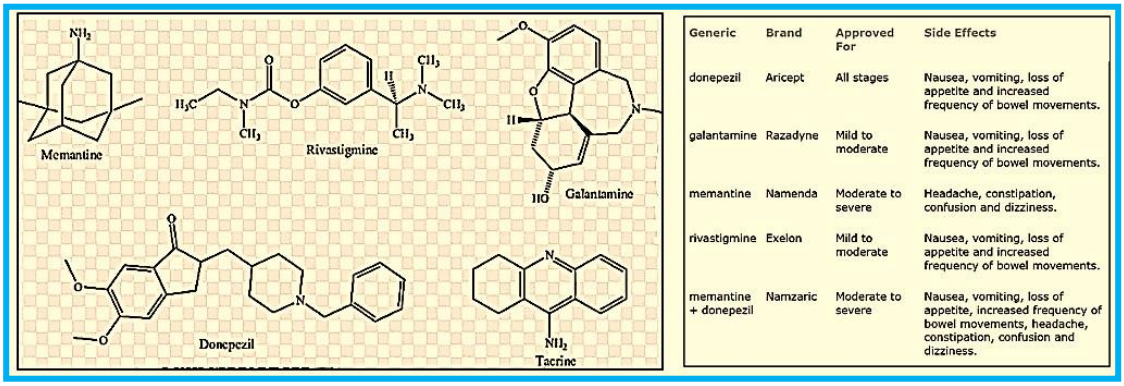
Shatzmiller and the group have identified a simple sequence of 5 amino acids in the structure of Dermaseptin S5, namely Lys-Ala-Ala-Ala-Lys A. This was the basis for the design, synthesis and evaluation of the surrogate's B and C whereas C contains a β -turn mimic as moiety.

There is a complicated situation regarding the mechanism of action in which AMP eradicate the bacteria. The Mitsuzali-Shai-Huang membrane disruption mechanism [88] has become the domain of not all the AMPs. Many may disintegrate membrane but it is clear today [3].

The way Surrogates corrupt the cell membrane may become a high hurdle in the process of AMPs to become the future antibiotic drugs. In their considerations. Researchers thought that membrane protein are not significant players in the eradication process. It might become the significant factors permitting the surrogates to enter the membranes complexes [89]. For gram-positive as well as for gram-negative bacteria. it seems that the way to the top is not so clear.

There is no cure for Alzheimer's disease [90]. Although current medications applied can not cure Alzheimer's or stop it from progress, they may help halt development of symptoms, such as memory loss and confusion, for a limited time.

The drugs below are not curing agents. They may be applied for a limited time and are palliative drugs.



Generic	Brand	Approved For	Side Effects
donepezil	Aricept	All stages	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
galantamine	Razadyne	Mild to moderate	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
memantine	Namenda	Moderate to severe	Headache, constipation, confusion and dizziness.
rivastigmine	Exelon	Mild to moderate	Nausea, vomiting, loss of appetite and increased frequency of bowel movements.
memantine + donepezil	Namzaric	Moderate to severe	Nausea, vomiting, loss of appetite, increased frequency of bowel movements, headache, constipation, confusion and dizziness.

FDA [91] approved drug substances.
They are palliative agents for limited time application

Targeting the Inflammation-Infection route in Neurodegeneration Remedy Research

The inflammation Hypothesis replaces the well-known Amyloid tract. The New look of neurodegeneration deals with gut and skin microbiome penetrating a leaky BBB to initiate microglia response, neuron inflammation and crawling infection of stroma and parenchyma of the brain.
The importance of increased permeability is that molecules that Usually cannot enter the brain to manage access, the inflammatory response is triggered as a result. Leaking brain is more sensitive to substances such as heavy metals, pathogen likes bacteria and viruses, environmental toxins and other harmful chemicals.
In the century of efforts to find cure and comfort in the neurodegenerative diseases of medicine, many approaches were applied [92] such as the CHOLINERGIC idea, OXIDATIVE STRESS theory, METAL IONS significance, the AMYLOID HYPOTHESIS, and the here discussed INFLAMMATION route.

The amyloid hypothesis guided many researchers and pharma companies. Many have tried to attack this disease by reducing amyloid plaques, but inflammation may be the real culprit. The Amyloid cascade idea is built on an assumption, which postulates the alternative modes of digestion by a membrane enzyme, secretase (a membrane conglomerate constructed of 4 or 5 subunits proteins). The protein Amyloid Precursor Protein (APP) is digested among other fragments, into the Amyloids. The Precursor Protein is not the only substrate for the secretaries. Other proteins like Insulin are also digested by the secretaries. The APP originates in the liver. It penetrates the BBB and reaches the membranal protease conglomerate. This cleavage of APP proceeds in an Abnormal damaging and Normal non-damaging ways. It produces many polypeptides, Amyloids that could a: (Antimicrobial peptides that can eradicate microbes), b: form aggregates in a self-assembly mechanism.

Those aggregates then from the aggregates of the amyloid fibrils, and higher supramolecular constructs that can eradicate the neurons, probably by destroying the synapses in the synapse "cluft" space. Kill neurons and cause neurodegeneration.
For almost 30 years this hypothesis was the leader of many scientific projects, it failed to bring remedy to the sick. Today, it is still considered but in a broader frame, based on the infiltration of microbes through damaged Blood-Brain Barrier, causing inflammation and neuronal infection which is demanding AMPs as immunosuppressive agents. Many proteins digested by proteases including Insulin, and those "saviors" turns to be the basis for aggregates, fibrils and more.
Continuous work in the search for disease change agents focuses primarily on screening molecules that can break down or inhibit the formation of proteins 'key' diseases, characterized by either β-amyloid in AD, synuclein-α in Parkinson's disease (PD) PrPSc infertility diseases such as Jacob's disease (Creutzfeldt Jacob disease) is a hallmark of brain degeneration [93]. The dual characteristics of Amyloid- β polypeptides is one of the causes of the neurodegeneration.

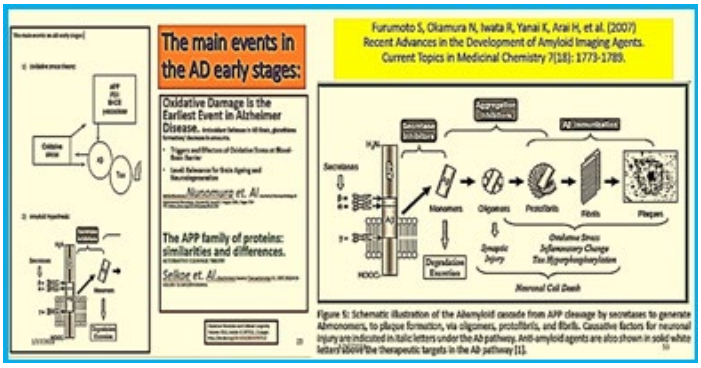
The New Inflammatory Hypothesis [94] : The Intrinsic Model

Currently, there are two new models of the inflammatory hypothesis of AD, intrinsic and external. The internal inflammation model constitutes the complete 'brain- blood' barrier (BBB) that restricts the entry of neurotoxic and systemic lymphocytes into the brain. As a result, the glial brain cells can produce a localized innate immune system when challenged by foreign agents. Historically, neuroinflammation has been primarily seen as a downstream of the amyloid hypothesis that the presence of amyloidogenic peptides leads to the activation of microglia that initiates pro- inflammatory steels and releases neurotoxic substances that may cause neuronal changes in neurons. GWAS now affects congenital immune genes as a risk factor and supports a significant role of AD inflammatory pathogens by an improper activation of a complement system in conjunction with neurofibrillary plates (NFTs).

A broad genome study (GWAS) is a great study to identify genetic variants of a trait, in particular to identify the relationship between singlepolymorphisms of nucleotides and common human diseases such as heart disease, inflammatory bowel disease, type 2 diabetes, and psychiatric disorders. The standard strategy for studies of population-based events testing for GWAS is described in this chapter. We provide an overview of the concepts behind GWAS, as well as provide guidance on the statistical methods used in GWAS [95].

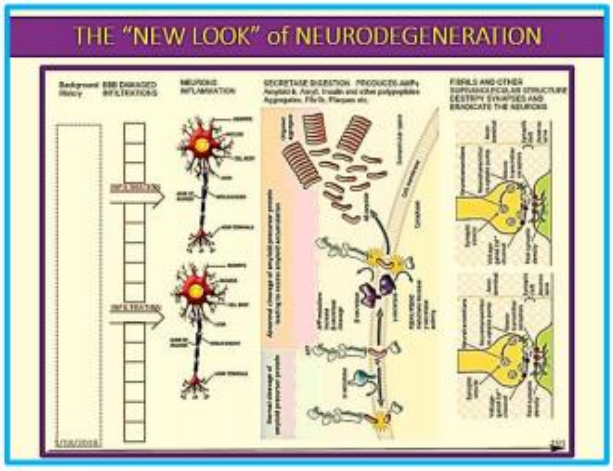
For three decades the Amyloid hypothesis [96] was leading in popularity compared to the oxidative stress ideas. It guided

researchers in the neurodegenerative diseases area. This idea contributed a lot to the progress of the respective quest for remedy, the amyloids, amylin, insulin and other antimicrobial agents [97] were held responsible for neuron death by their aggregates, fibrils, and plaques [98]. However, it seems today that the culprit is not there. On the other hand, more and more evidence is accumulating to favor an idea that was almost abandoned, the microbial inflammation [99] and infection [100] as the onset and significant cause of neuro-degeneration and death.



The "OLD" Look of Neurodegeneration

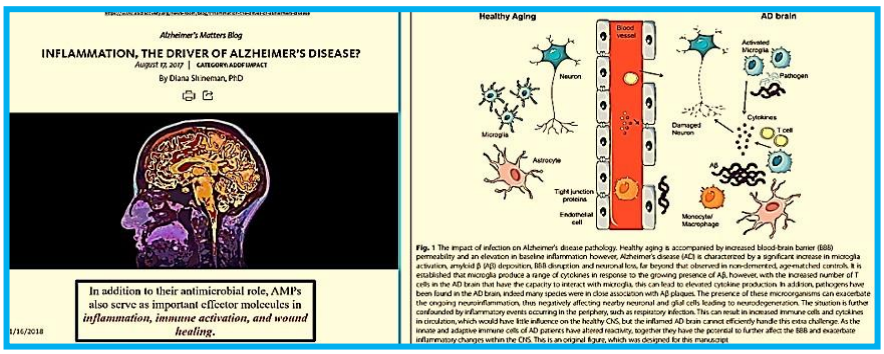
Could it be that neurodegenerative primary disease, the Alzheimer's disease stems from the toxic remnants of the brain's attempt to fight off infection?



The Inflammation-Based Neurodegeneration, New "Look"

Provocative new investigations by a team of researchers at Harvard leads to this fantastic hypothesis, which can explain the sources of plaques, the mysterious and challenging hard pills that pockmark the brains of people with Alzheimer's.Researchers report on various infections as possible triggers of neurodegeneration of the brain parenchyma.

The scientists report a scenario seemingly out of science fiction. A virus [101], fungus or bacterium [102] enters the brain, passes through the membrane a blood-brain barrier it becomes leaking as people age. The brain's defense system hastens to stop the intruder by creating a sticky cage of proteins called β amyloid. The bacterium, like a spider web spider, is trapped in a cage and perishes. The left behind is the cage a sign that is the hallmark of Alzheimer's?



Microbiome Microbes Crossing the Blood-Brain Barrier into the Parenchyma and Stroma of the Inner Brain

In recent years, The Amyloid Precursor Protein -Secretase-Amyloid-hypothesis is losing momentum. Instead, inflammation [103]. Inflammation is a defense response against various insults, designed to remove toxic substances to inhibit their harmful effects. It has a collection of a dazzling array of molecular cellular mechanisms and a complex network of controls to keep them in check. In degenerative diseases, inflammation may be triggered by an accumulation of proteins with abnormal conformation or by signals arising from injured neurons. Given the number of functions of many inflammatory factors, it was difficult to pinpoint their role in specific pathological conditions. Neurological infection [104], it takes the lead in the quest to understand the cause of the many neurodegenerative diseases [105]. When the BBB [106] is damaged, gut bacteria, protein and other" prohibited" bodies can enter the subconscious brain and start the process, that continues for decades, of neuronal eradication, and finally interception and death [107].The quest for remedy is reaching every corner. Here are some examples of agents discovered in the plants [108].

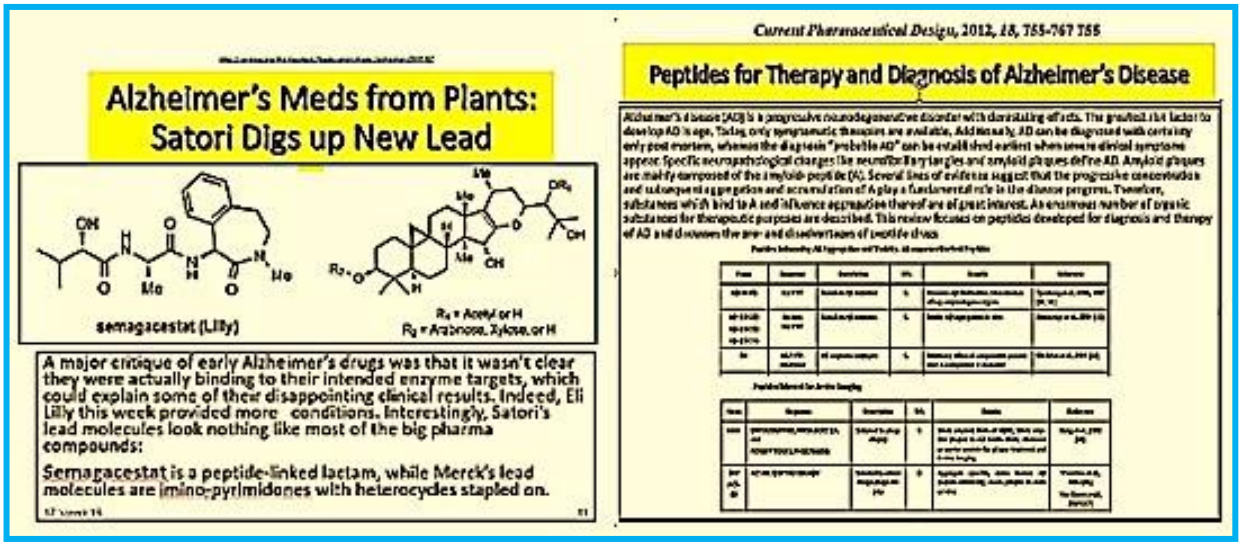
The leaks in the 400 miles of blood vessels that surround the brain, is hard to locate in the living brain, which makes a possible medicinal drugs treatment very difficult to the practically impossible today. Recently, nutritional measures suggested in many internet publications on how to fix a leaky BBB [109]. These do not deter researchers from seeking instrumental methods, like LASER [110] based approach for the initial instrumental diagnosis of neurodegeneration.

The New Inflammatory Hypothesis [111] : The Intrinsic Model

Currently, there are two models of the inflammatory hypothesis of AD, intrinsic and external. The internal inflammation model constitutes the complete 'brain-blood' barrier (BBB) that restricts the entry of neurotoxic and systemic lymphocytes into the brain. As a result, the glial brain cells can produce a localized innate immune system when challenged by foreign agents. Historically, neuroinflammation has been seen mainly as a downstream of the amyloid hypothesis that the presence of amyloidogenic peptides leads to the activation of microglia that initiates pro-inflammatory steels and releases neurotoxic substances that may cause neuronal changes in neurons. GWAS now affects congenital immune genes as a risk factor and supports a significant role of AD inflammatory pathogens by an improper activation of a complement system in conjunction with neurofibrillary plates (NFTs).

Curing Alzheimer's is there any potential Remedy?

New approaches in AD drug discovery



Innovative Approaches in Neurodegeneration Drug Discovery

Once inflammation is on, it immortalizes herself. These inflammatory cytokines spread throughout the body causing stress and oxidation. Oxidative damage is the earliest event in Alzheimer's disease [112]. Destroy the fragile machines of the tissues and the mitochondria, especially. In the brain, inflammation is used to replace the use of tryptophan towards the production of anxiety-stimulating substances like quinoline, instead of toward serotonin and melatonin. They produce a recurring set of symptoms called "disease syndrome," noting for overlap with "depressive" symptoms: fatigue, sleep disorders, decreased social activity, mobility, libido, learning, anorexia, and anhedonia. Psychiatric investigations have observed that patients with higher levels of inflammatory markers (such as CRP and C-reactive protein) are less likely to respond to antidepressants. At the same time, more likely to respond to anti-inflammatories [113a,b], For example, some AMPs alter the properties of the mammalian membrane or interact with its receptors to influence diverse cellular processes including cytokine release, chemotaxis, antigen presentation, angiogenesis and wound healing. Today, we are beginning to see that much of the importance of AMPs in mammals might lie in their multifunctional role. However, increasing evidence indicates that some AMPs can confer protection by an indirect mechanism and not merely because they can kill microbes. They can function as potent immune regulators, altering host gene expression, acting as chemokines and inducing chemokine production, inhibiting LPS

or hyaluronan- induced pro-inflammatory cytokine production, promoting wound healing and modulating the responses of dendritic cells or T cells of the adaptive immune response. In this way, AMPs are acting as a bridge between innate and adaptive immunity. These functions favor resolution of infection and reverse potentially harmful inflammation, and complement the direct antimicrobial action. Here, we review some of the essential functions [114] of AMPs that are not related to their direct antimicrobial action.

Ultrashort SMAMPs (Synthetic Mimics Antimicrobial Peptide) [11], can become very instrumental in neuro-medicine. We have demonstrated that SMAMPs (mimics of 5 amino acids), based on some privileged scaffolds like 1,4 -DHP and Azepine cross the BBB [115], and enter the Stroma and Parenchymas of the living brain. There is currently an immediate need for improved biomarkers [116] due to growth in numbers of affected people and in disease severity and therapies for psychiatric, developmental, traumatic, inflammatory, infectious and degenerative nervous system disorders. These, in whole or in part, are a significant societal burden.

Lost productivity of the patient and his or her caregiver and the emotional and financial burden cannot be overstated. The need for improved health care, treatment and diagnostics are immediate. What a means to such an end is nanotechnology. Indeed, recent developments in the field of health that allow nanotechnology and nanomedicine range from biomarker detection, including brain imaging to therapeutic applications of neurodegenerative, inflammatory, and harmful nervous system disorders.

Role of Antimicrobial Peptides (AMPs) and Synthetic Mimics of Antimicrobial Peptides (SMAMPs) in Brain Inflammation

Most cationic peptides, such as β-Defensins, assigned initially to a microbial function are now recognized as mediators of innate immunity and adaptation. The following supporting evidence is presented for the hypothesis that neuropathological changes associated with chronic disease conditions of CNS involve abnormal expression and regulatory function of specific antimicrobial peptides. It is also suggested that these changes exacerbate the pro-inflammatory conditions in the brain, which ultimately intensify the neurogenerative process [117].

How can antimicrobial peptide surrogates contribute to neurodegeneration research?

- ✓ Inflammation is back; AMP are anti-inflammatory
- ✓ Foldamers as secretase inhibitors [118].
- ✓ Antimicrobial peptides surrogated do not aggregate.
- ✓ Immunotherapy
- ✓ The unique mechanism of inhibition of -secretase. The function of Notch in
- ✓ γ-secretase inhibitor) GSI)
- ✓ Aib (2-amino isobutyric acid) aided BBB penetration of probes and drugs

We would like to offer the investigation of some leading STAMPS as neurodegeneration retarding compounds.

In addition to the role of anti-microbial, AMPs also act as essential effector molecules for inflammation, immune activation, and wound healing. Antimicrobial peptides are needed to fight neuroinflammation.

Antimicrobial peptides are part of the innate immune system of many organ systems, but little is known about their expression and function in the brain. Antibacterial cathelicidin AMP in rats (human homologate LL-37) not only exhibits intense bacterial activity but also functions as a chemoattractant for immune cells. AMPs participate in brain immunity by stimulating the production of cytokines and cell activation and glia to help protect brain cells by causing neurotrophic factors. AMPs are essential components of innate CNS immunity which acts to protect brain cells by causing neurotrophic factors.

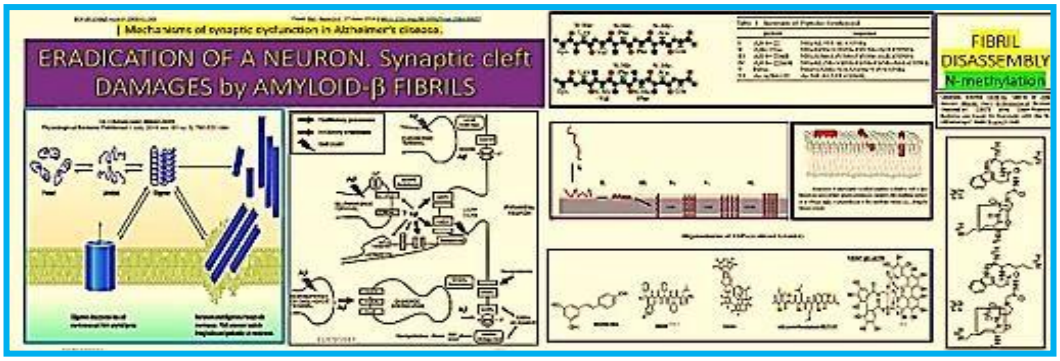
Antimicrobial peptides in neurons are significant players in neurodegenerative disease inhibitors. β-Defensins have significant tasks to protect the parenchyma brain lobes. Amylin AMPs (sugar metabolism) and amyloid β, and others like insulin(diabetes) have a significant role in inhibiting the spread of inflammation and infection by their involvement in the harness of immune vaccine agents as follows:

The human β-defensins [119] (hBDs) are a canned family of antimicrobial action and immuno-stimulatory the peptides operate, at first, by epithelial cells in response to invasion by bacteria, fungi, and certain viruses. To date, the most studied research members of these peptides are HBD-1, -2 and -3. Expression of HBD-1 and 2 has been demonstrated in the past microglia culturally astrocytes of both mouse and the human brain. Unlike HBD-2 and 3, HBD-1 is constructively expressed and is not felt by pro-inflammatory factors.

Anti-Neurodegradation Agents-Antimicrobial Peptide Surrogates

Recently, publications [120] to deal with the potential contribution of pathogenic bacteria to AD aging. Bacteria and bacteria and other proteins enter a defective brain blood barrier (BBB) that attacks the neurons that cause inflammation in the brain. This inflammation, which is considered a primary step in the development of fatal diseases: Alzheimer's, Parkinson's and dozens of other known diseases in the brain.

Natural immune systems apply to microbial peptides, β-defensins [121]. For example other such agents as amylin, β-amyloid (Aβ) are "harnessed" to protect the neurons, to stop inflammation. These microbial materials bring with them a lousy trait: they form aggregates, fibrils, tangles and other supramolecular structures. Theses in the brain and inflammation have caused the bodies to hit the most sensitive areas of neurons in the brain, the synapses. To bring about degeneration and death by damaging the synapse membrane in microbial form; Dig holes in the cell membrane.



Damaging Fibrils in Neurodegeneration

Antimicrobial polypeptides are useful in the cure of neuronal injuries. An example is Antimicrobial peptides and complement in neonatal hypoxia-ischemia induced brain damage [122]. It is well known that HI (hypoxia, ischemia) injury to the brain associated with infiltration of inflammatory cells to the brain. Neutrophils are the abundant type of leukocytes and are an inseparable part of the innate immune system. Although adult rodent testing of ischemic insult suggests that neutrophils are known to accumulate in the brain as early as 4-6 hours post-injury, and lasts up to 48 hours. This does not look to be the case for neonatal HI injury, where neutrophil infiltration into the injured brain is less marked, with a smaller number present at 42 hours post-insult. However, this study showed that P7 neutropenic rats had a 70% reduction in brain swelling at 42 h after HI compared to littermate controls. Therefore, despite the suggestion that neutrophils do not accumulate in the undeveloped brain following HI, they still play an important role in worsening of neonatal brain damage.

Although AMPs are best known for their anti-bacterial properties, a large number of them also possess chemotactic properties. α-defensins are chemotactic for PMNs and T cells, HBDs for monocytes, DC, and CD4 T-cells, while LL-37 are chemotactic for granulocytes as well as CD4 T cells. All this indicates the essential role of AMPs as a link between immunity Homeland adaptation. AMPs have antimicrobial properties, but they are also an essential part of the inflammatory response, and various environmental stimuli are involved in multiple signaling pathways to promote their synthesis. Pro-inflammatory molecules (IL-1, TNF-α, IL-6) and bacterial products increase the expression of cathelicidin and defensins by activating signaling pathways AP-1, JAK2, and STAT3. Overall AMPs appear to be an essential component of antimicrobial host defense, directly disabling the pathogens and contributing to the immune response associated with pathogen removal.

"Here, we synthesize research behind the emerging hypothesis that inflammation-which can result, for example, from viral or by entering the parenchyma through a leaky BBB to initiate a microbiome induced infections-can initiate and propagate chronic neuronal dysfunction, an event that precedes the clinical onset of many neurodegenerative diseases. Therapeutic approaches that target immunological pathways in the prodromal phase of diseases might decrease the incidence of neurodegenerative disorders and increase the therapeutic window for neuroprotection".

Research Perspectives

Based on our previous results on ultrashort peptide surrogates, we would like to proceed in three main directions:

1. 1,4-Dihydropyridine based STAMPS
2. Azepine based STAMPS
3. N-Methyl β -turn mimics STAMPS.

DHP Penetrates BBB through DHP Receptors

We Investigate L-type calcium channel blockers of the dihydropyridine class for association with Parkinson's disease because these drugs traverse the blood-brain barrier [123], are potentially neuroprotective, and have previously been evaluated for impact on PD risk.

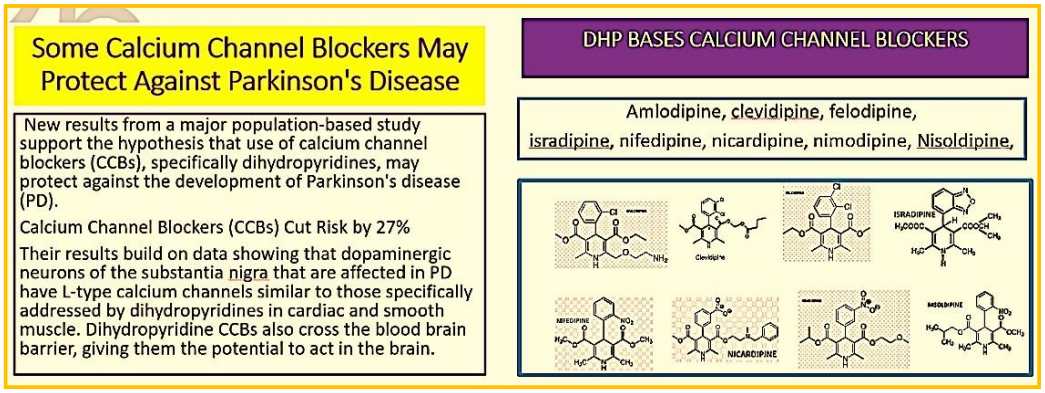
Calcium signaling in Parkinson's disease [124] Calcium (Ca2+) is a universal second messenger that regulates essential activities of all eukaryotic cells.

We synthesize a new broad antibacterial spectrum Cationic peptidomics focuses on hydrophobic 1,4-dihydropyridine (1,4-DHP) scaffold. Synthesis involves Preparing the scaffold in three steps Hantzsch reaction followed by simultaneous coupling of The 1,4-DHP scaffold to two cation-bearing dipeptides Sidechains. Synthetic pep Red blood cells are mammalian. The compounds were found Antibacterial activity against Gram (-) and Gram (+) Bacteria with MICs in the range of 35-100 mg/mL. Goddess Minor microbiological peptides and mathematicians will lead to more Effective anti- bacterial drugs are synthetically based Accessible scaffold.

It is of critical importance to neurons, which have developed comprehensive and complex pathways to pair Ca2 + signal to their biochemical machines.
In particular, Ca2 + participates in transmitting the depolarizing signal and contributes to synaptic activity. In the course of aging and the processes of neurodegenerative diseases, the ability of neurons to maintain an adequate level of energy may be impaired, thus affecting Ca2 + homeostasis.

In Parkinson's (PD), many signs of neurodegeneration develop from the mitochondrial function that is damaged by specific

damages of toxins on the mitochondrial respiratory chain and/or genetic mutations. Although these effects are present in almost all cell types, the distinguishing feature of PD is extreme selectivity of cell loss, which is limited to dopaminergic neurons in the central part of the nigra pars compacta material.



Dihydropyridine Based drug-like Molecules in Neurodegeneration Research

[125] Reached phase III candidate for the treatment of Parkinson's disease.

The agent ISRADIPINE

A new design of a Phase III trial of Isradipine in early Parkinson disease (STEADY-PD III), this study examines isradipine as a potential new neurotoxic agent in the PD based on reliable strong preclinical data.

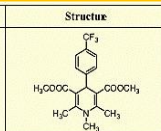
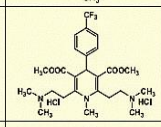
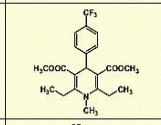
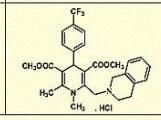
The study design STEADY-PDIII is unique in assessing the effect of isradipine over 36 months, at a time when all participants will be on ST, which allows us to determine whether the benefit is sustained "on" traditional symptomatic therapy (ST). In addition, the study design enables us to determine whether the effects on motor function are corroborated by important secondary outcomes that have evaluated clinically relevant measures of ST use, motor complications, non-motor functioning, global disability, quality of life, ambulatory capacity, and cognition. This design is innovative and innovative and enables the determination of long-term benefits on a number of clinically relevant results in a relatively small group on the derived ST benefit.

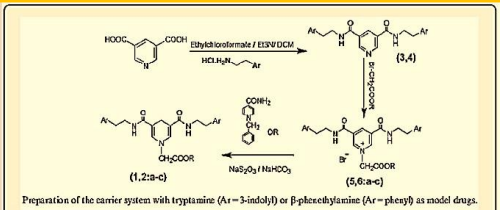
It is reported that Some Calcium Channel Blockers May Protect Against Parkinson's Disease. L-type channel: Studies [126] on short Aβ peptides provided an early indication of involvement of calcium channels in the Aβ pathology. Application of Aβ25–35 to cultured neurons caused cell degeneration, which was prevented by nimodipine.

Overall, the current data suggest that use of calcium channel blocker antihypertensive, significantly slows the rate of progression of subjects to dementia compared to those subjects who do not use CCBs and that the potential protective effects on cognitive decline might mediate through modulation of proteins involved in Aβproduction. The current data coupled with existing risk assessment studies suggest that use of antihypertensive (AHTs) may significantly alter AD risk/progression and that use of these drugs should be considered in clinical trials of anti-AD therapeutics.

Dihydropyridines Modulate Heat Shock Responses and have a Neuroprotective Effect in a Transgenic Mouse Model of Alzheimer's Disease [127]

Heat shock proteins (Hsps) have accompaniment activities that play a significant role in the homeostasis of proteins by preventing misfolding, by clearing the accumulated and defective proteins from the cells, and by keeping the proteins in an active state. Alzheimer's (AD) is thought to be caused by amyloid-peptide that activates tau hyperphosphorylation, which is neurotoxic. Although proteostasis capacity decreases with age and facilitates the expression of neurodegenerative diseases such as AD, upregulation of companions improves prognosis. The target of our study is to identify HOS-strong producers inducers that strengthen protein homeostasis for AD treatment, particularly 1,4-dihydropyridine derivatives optimized for their ability to regulate cellular voltage responses. Based on actual toxic data and HSP- stimulating activity, LA1011 was selected for in vivo analysis of its neuroprotective effect in the APPxPS1 mouse model of AD. Here, we report that six months of administration of LA1011 effectively improved learning and memory spatial functions in wild-type mice and eliminated neurodegeneration in double mutation mice. Furthermore, HSP co-treatment inducer preserves the number of neurons, increases dendritic spine density, and reduces tau pathology and the formation of amyloid plaque in transgenic AD mice. In conclusion, the HSP Co-Inducer LA1011 is a neuro-purposive and is therefore a potential pharmaceutical candidate for the treatment of neurodegenerative diseases, particularly AD.

Code	Structure	Chemical Name
BAY w 9798		Dimethyl 1,2,6-trimethyl-4-(4-methylphenyl)-1,4-dihydropyridine-3,5-dicarboxylate
LA1011		Dimethyl 4-(4-trifluoromethylphenyl)-2,6-bis-(2-dimethylaminoethyl)-1-methyl-1,4-dihydropyridine-3,5-dicarboxylate dihydrochloride
LA1030		Dimethyl 2,6-diethyl-1-methyl-4-(trifluoromethyl-phenyl)-1,4-dihydropyridine-3,5-dicarboxylate
LA1044		Dimethyl 2-(1,2,3,4-tetrahydro-1H-isoquinolin-2-ylmethyl)-1,6-dimethyl-4-(4-trifluoromethyl-phenyl)-1,4-dihydropyridine-3,5-dicarboxylate hydrochloride



Preparation of the carrier system with tryptamine (Ar = 3-indolyl) or β-phenethylamine (Ar = phenyl) as model drugs.

Figure 13: Preparation of Pyridinium model for BBB crossing [61].

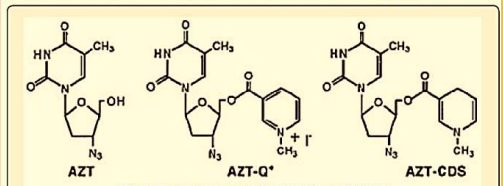


FIG. 1. Chemical structures of AZT, AZT-Q+, and AZT-CDS.

Figure 12: antiviral pharmaceuticals designed to cross the BBB [60].

"Trojan Horse" Introduction of Active Agents into the Brain DHP as Leader [128]

Brain infections and the passage of the BBB brain blood barrier. Proper management of central nervous system infections (CNS) requires antimicrobial substances to penetrate the blood-brain barrier (BBB) and to obtain concentrations in the central nervous system that are suitable for the elimination of the pathogen contaminant polypeptides as Medicine for Parkinsonism and Autoimmune diseases. Use of Botulinum neurotoxin [129,130] type A (BoNT/A0 injections in case of Parkinsonism. Most physicians are generally aware of Parkinson's disease, they often are not familiar with dystonia.

Botulinum toxin is a very effective treatment for symptoms associated with many disorders. People with abnormal arm, leg or trunk positions, or one of the other movement disorders such as dystonia, blepharospasm, or mimicial spasm may be of dramatic benefit with appropriate injections. Botulinum toxin treatment by injections may also be used to treat excessive mucus, chronic migraine headaches, and spasticity.Botulinum toxin blocks the neurotransmitter and acetylcholine loose into the space between muscles and nerves. This temporarily reduces muscle contraction and allows the patient to develop a more functional, healthy posture.

Some patients who experience dystonia-related pain may also experience improvement.

There are four toxins approved in the USA, and in the Department of Diseases and Diseases of the Northwestern Parkinson University we have access to all approved toxins and experienced doctors who can inject.

Although the drug is a toxin, proper penetration of the muscles at the right doses usually provides the desired benefit with little risk, although your doctor will discuss possible complications of treatment before proceeding.

Possible signs of botulinum toxin injection include:

Blepharospasm, Spasmodic Contraction, Dystonia of the Neck, Chronic Migraine, Spasticity, Sialorrhea (mucus)

As one of the collective movement disorders seen in general practice, many family and population studies have suggested that as many as two-thirds of patients with dystonia may be underdiagnosed and it is likely that misdiagnosis occurs frequently. Moreover, there is little information on the prevalence of dystonia. Dystonia is a neurodegenerative disorder syndrome in which continuous or recurrent muscle spindles return and lead in repetitive movements or in abnormal posture.

The movements may resemble shivering. Dystonia is often intensified or worsened by exercise, and symptoms may progress into adjacent muscles.

The disorder may be of genetic origin or caused by other factors such as birth or other physical trauma, infection, poisoning (eg lead poisoning) or drug response, especially neuropathy. Treatment must be specifically tailored to the needs of the individual and may include oral medications, botulinum injections, neurotoxin injections, physiotherapy and/or other supportive treatments, and/or surgeries such as deep brain stimulation.

Injections of botulinum toxin a are a useful treatment for sialorrhea (is excessive production of saliva) in Parkinson's disease (PD). Based on relatively high rates of dry mouth appears with botulinum toxin B, there is a reason to suspect that this may also improve sialorrhea.

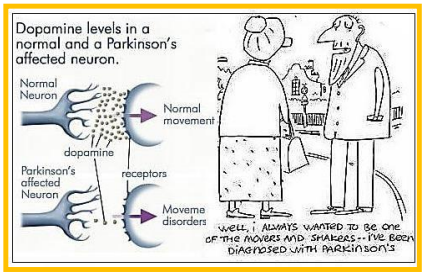
Botulinum type A neurotoxin A (BoNT/A) is one of the most potent toxins known as a potential biological threat. At the same time, it is one of the most common therapeutic proteins used every year by many people, especially for cosmetic purposes. Currently, its clinical use of certain types of pain is increasing, and the long duration of the effects represents an exceptional clinical value. Repeated injections of BoNT-A are safe and effective in treating sialorrhea in patients with PD. Based on tests results, it seems that there is a maintenance of efficacy after a three-year period and an increase in the mean duration of efficacy with the number of injections.

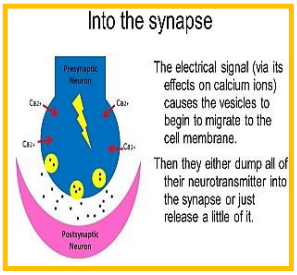
Most Parkinson's drugs aim at the synapse cleft to better promote acetylcholine traffic.

Parkinson's Disease: Understanding Your Medications

Medicines are key to treating Parkinson's. You may be prescribed one or more medicines. Be sure you know the names of your medicines and when and how to take them. Ask your healthcare provider what side effects you might expect. Also ask if you should avoid eating certain foods or drinking alcohol.

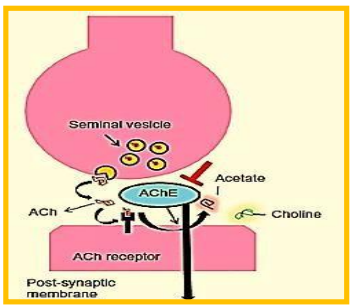
Types of medicines*	Examples	How they help
Levodopa combined with carbidopa	Carbidopa-levadopa	Levodopa replaces missing dopamine. Carbidopa helps levodopa enter the brain with fewer side effects.
Dopamine agonists	Pramipexole, bromocriptine, ropinirole, rotigotine	Imitate the way dopamine works in the brain.
MAO-B inhibitors	Selegiline, rasagiline	Help dopamine work longer.
COMT inhibitors	Entacapone, combination of carbidopa, levodopa, and entacapone	Taken with levodopa. Help dopamine enter the brain and work longer.
NMDA antagonists	Amantadine	Reduce involuntary movements and tremors.
Anticholinergics	Trihexyphenidyl, benztropine	Reduce tremor.

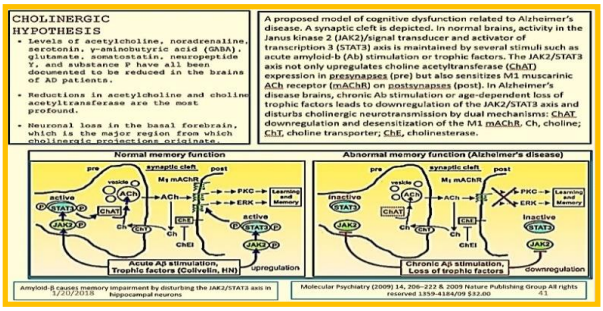




https://www.mountnittany.org/articles/healthsheets/5385
http://scicurious.scientopia.org/2010/08/23/back-to-basics-1-neurotransmission

Ca++ function in the synapse is affected by**1,4-Dihydropyridines,** this influences the acetylcholine traffic between the two regions of the synapses and better traffic produces less neuronal dysfunction.

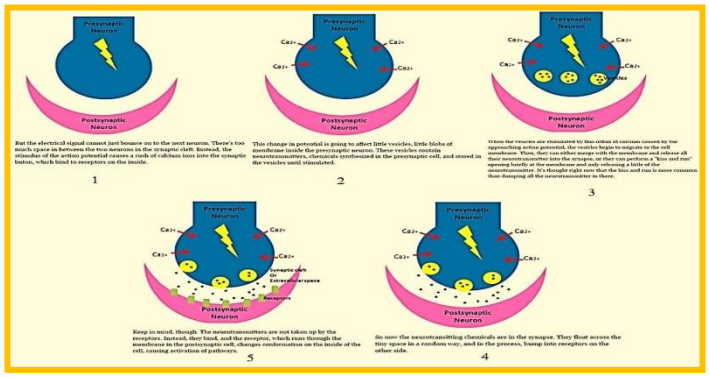




Our work attracts many organizers of conferences for its potential in this area for example:

"We are honored to announce that the 2nd International Biotechnology Congress (IBC-2018) will be held during October 16-18, 2018 in Fukuoka, Japan. We are formally welcome you to propose an oral presentation in the field of 1,4-Dihydropyridine Cationic Peptidomimetics with Antibacterial Activity in the Stream 506: Pharmaceutical/Medical Biotechnology of the Congress."

"Three out of the five existing drugs-donepezil, galantamine, and rivastigmine-are from the class of drugs called choline inhibitors, which prevent the breakdown of a chemical messenger in the brain that is applicable for learning and memory. The fourth drug, memantine, regulates the activity of another chemical messenger in the brain, it is essential to learn and to remember. Both types of drugs help manage symptoms but act in different ways. The fifth drug is a combination of one of the cholinesterase inhibitors with memantine.



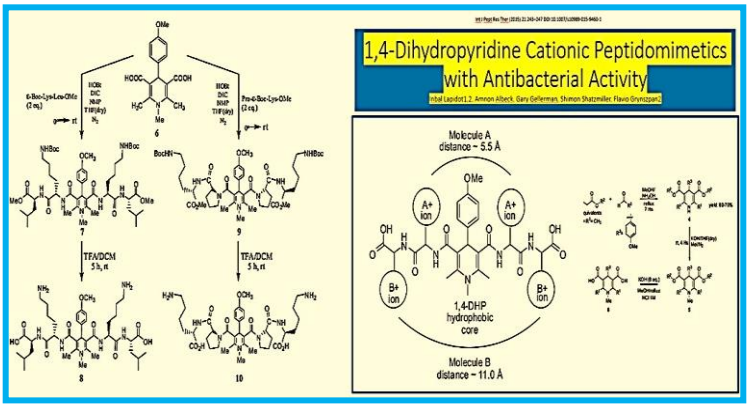
A Mediated Transfer of Neuronal Signal in the Synapse (115)

1,4-Dihydropyridine Cationic Peptidomimetics with Antibacterial Activity [131]

We broadly synthesized broad-spectrum antibacterial action and peptidomics on a 1,4-dihydropyridine hydrophobic scaffold (1,4-DHP). Synthesis consists of the preparation of the scaffold in the retaliation reaction in three stages, followed by simultaneous coupling of a 1,4-DHP scaffold to two side-bonded dipeptides.

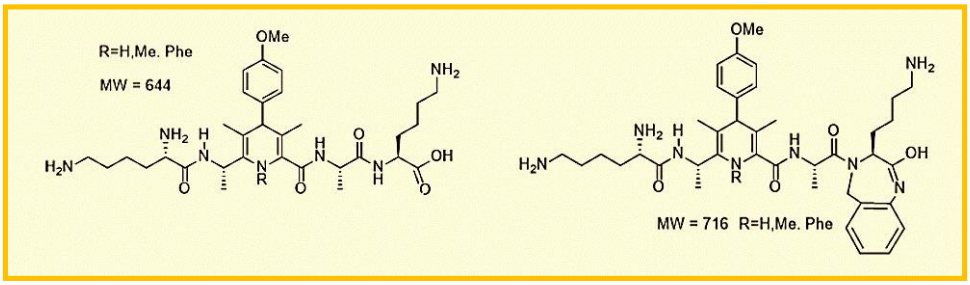
Synthetic peptidomics has no measurable hemolytic factor against mammalian red blood cells. The compounds were found to have antibacterial activity against Gram - (-) and Gram - (+) bacteria with MIC in the range of 35-100 mg/mL. These microbial cation peptidomimetics will lead to candidates for more effective antibacterial drugs based on a synthetic accessible scaffold.

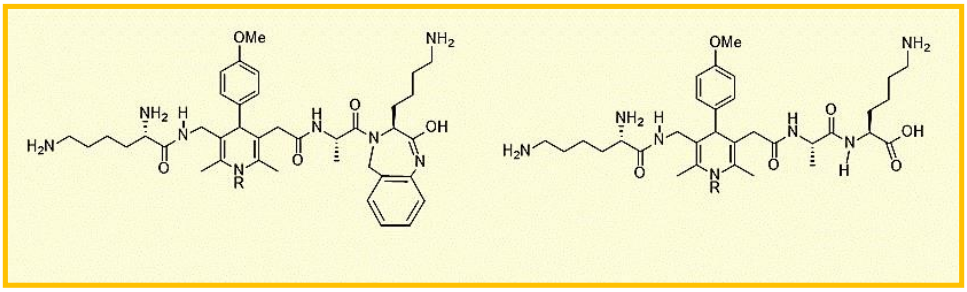
In addition to the role of anti-microbial, AMPs also act as effector molecules for inflammation, immune activation, and wound healing.



SMAMPs Generated and Tested in our Laboratory

Targets: Based on the Lys-Ala-Ala-Ala-Lys active pentapeptide isolated as a fragment of Dermaseptin S4.

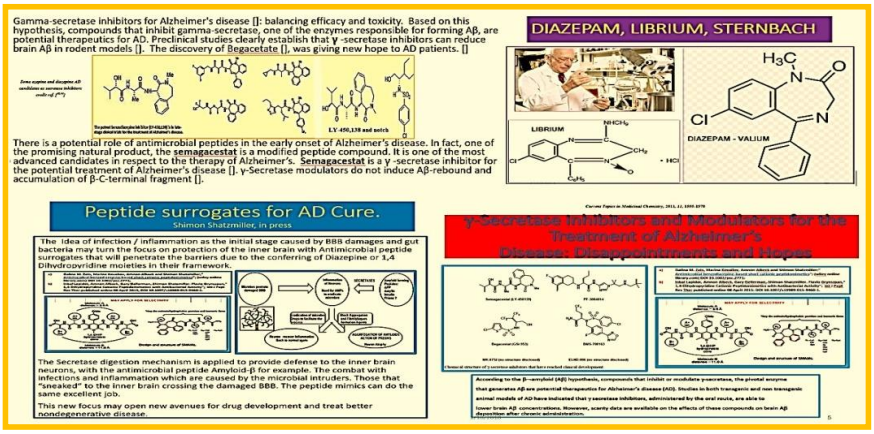




The suggestion of novel DHP bases SMAMPs.

Diazepam Based Smamps

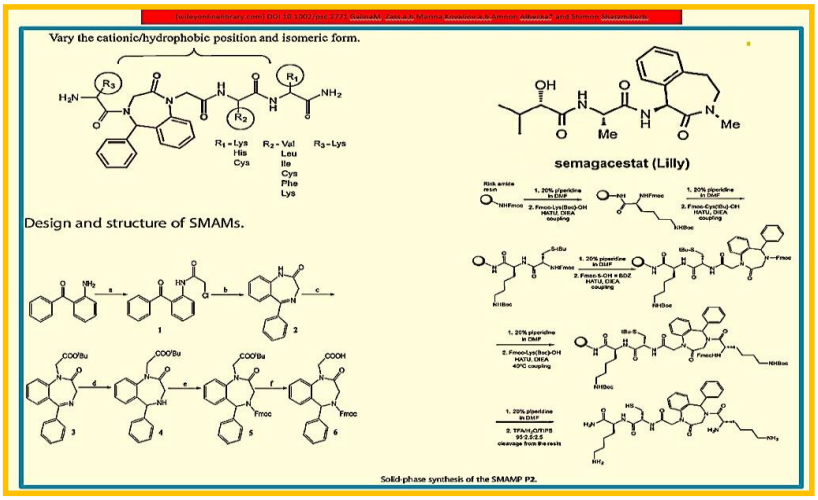
Since the findings of Sternbach, Librium, Valium and many more azepine based neurological Drugs, the Azepine unit has become most applied in the area.



AZEPINE Based Bioactive Agents

Many agents directed at inhibition of γ -secretases are based on azepine units. It might be a wish to mimics features present in the phase III candidate semagacestat. There is a potential role of antimicrobial peptides in the early onset of Alzheimer's disease. In fact, one of the promising natural product, the semagacestat is a modified peptide compound. It is one of the most advanced candidates with respect to the therapy of Alzheimer's. Semagacestat is a γ-secretase inhibitor for the potential treatment of Alzheimer's disease. γ-Secretase modulators do not induce Aβ-rebound and accumulation of β-C-terminal fragment. Our synthesis of diazepine based STAMPS is as follows:

The diazepine unit integrates 2 amino acid rests of the Lys-Ala-Ala-Ala-Lys (a unit of DermaseptinS4) rests. Since very potent compounds were identified in this series, e would like to extend this work to more analogs that will be tested as AMP surrogates on both G+ and G- bacteria and than as secretase inhibitors in the neurodegeneration field. The robustly supported synthesis will produce many compounds libraries for the biological activity tests.

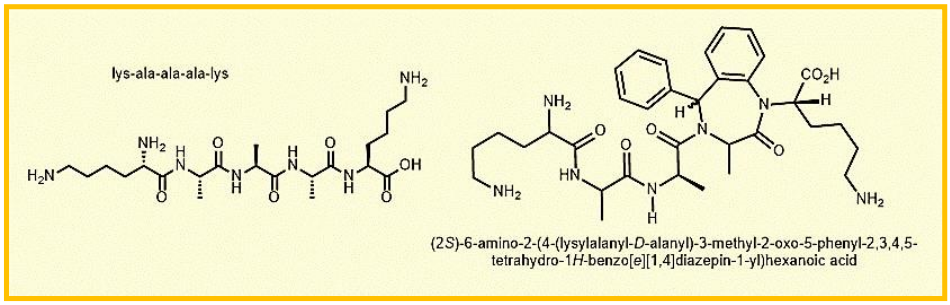


Solid State Resin Supported synthesis of azepine based bioactive compounds.

The Pentapeptide will be converted to the diazepine based molecule where constraints are introduced by the diazepine β -turn mimic as a moiety. A robustly supported synthesis may provide many analogs to be tested for biological activity.

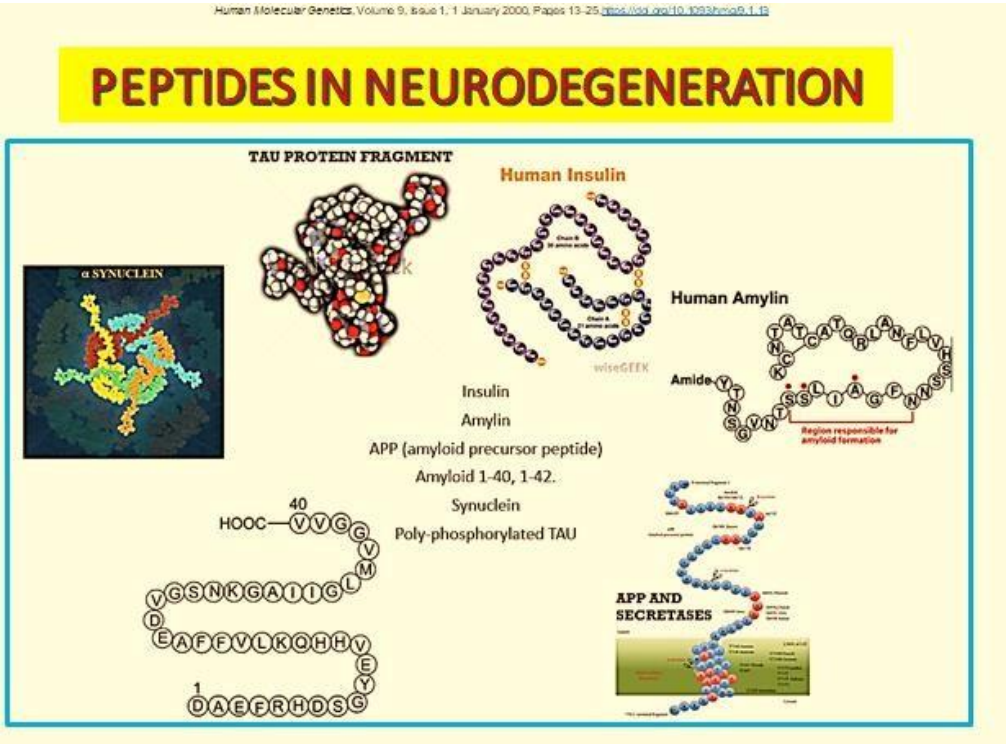
Antimicrobial peptides (AMPs) or their surrogates appear to be good candidates for developing new antibiotics. We describe here Synthesis of peptidomimetic compounds based on benzodiazepine scaffold surrounded by positive and hydrophobic positive amino acids. These compounds mimic the most suitable properties of cationic amps. The new design has benzodiazepines Scaffold consists of two amino acids glycine which gives a hydrophobic, 'hydrophobic' back flexibility, and two. The weapon is used for further synthesis on a solid phase for a combination of hydrophobic amino acids loaded. This approach has enabled. We have a better understanding of the effect of these properties on antimicrobial activity and selectivity. A new compound was discovered which has MICs of 12.5 μg/ml against Staphylococcus aureus and 25 μg/ml against Escherichia coli, similar to well-known Microbiological

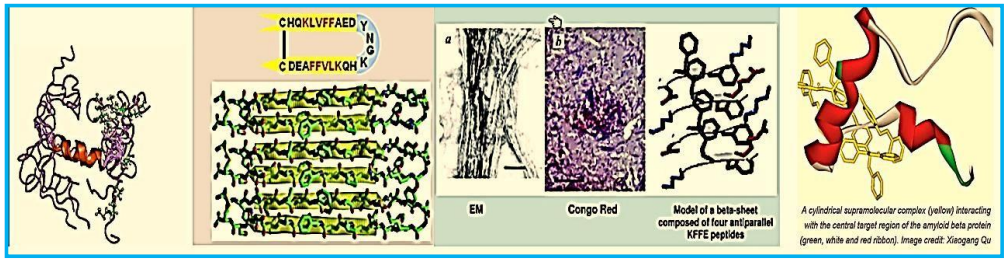
peptide MSI-78. Unlike MSI-78, the compound has a lower lytic effect Red blood cells are mammalian. These peptidomimetic compounds will pave the way for the future design of convincing synthetic imitations Of AMPs for biomedicine therapeutic applications.



Suggested azepine based SMAMPs.

Poly N-methylated peptide surrogates as selective antimicrobial agents.

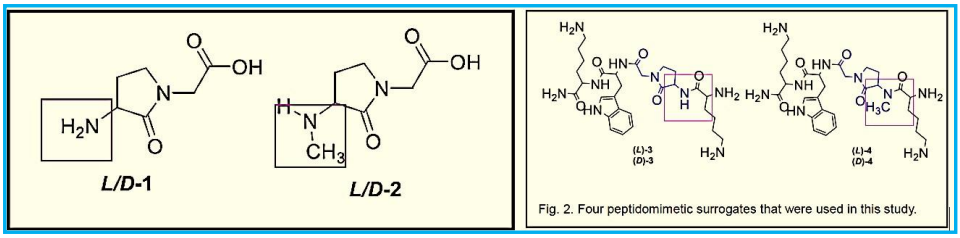




Amyloid Fibril Formation an Inhibition based on the KFFE unit.

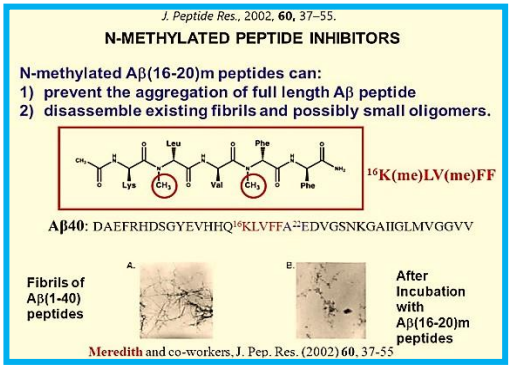
Introduction

N-methylation [132] is a useful structural change since it changes some of the main features needed for biological activity. The architecture and micro-Structural determinants of antimicrobial activity in Synthetic antimicrobial [133] peptide surrogates (SMAMPs), which mimic host defense peptides include charge, amphipathicity [134], hydrophobicity [135], flexibility [136] and H-bonding capacity [137,138]. We have published [139] on the preparation and conferring modified Friedinger lactams (β -turn mimics) N-H and N-CH3 analogs to short peptide sequences altering their ability to eradicate Gram-positive (Staph. Aureus) and Gram-negative (E. Coli) bacteria.

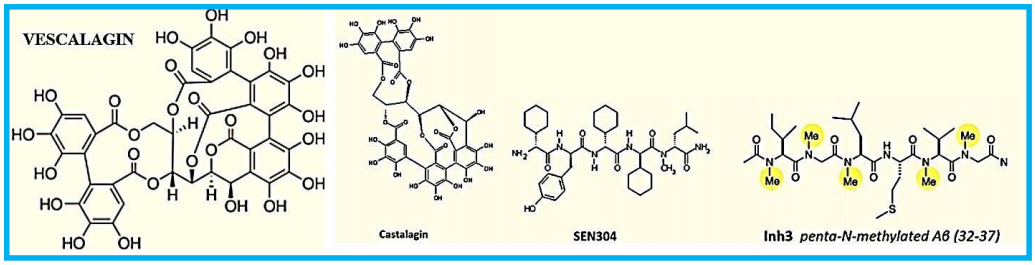


Modified Freidinger Lactam Based Stamps

The effect of the one N-Me modification showed in a comprehensive study a selectivity in eradication mainly expressed in the Gram-positive bacteria eradication. Poly N-methyl peptides were examined as aggregation Inhibitors of Amyloid-β (Aβ) [140].



Experimental and theoretical means [141] have demonstrated that the most common forms of the peptide, A β (1-42) and A β (1-40), self-assemble into amyloid fibers by a nucleation-condensation polymerization mechanism. The aim of this is to reveal the modes of action of three potential inhibitors at an atomic level of detail, with the ultimate goal of discovering new therapeutic agents reducing Aβ 42 toxicity. Methods: Thioflavin T (Thu) fluorescence assay, Atomic Force Microscopy (AFM), nuclear magnetic resonance (NMR), MTT assay is a colorimetric assay for determining cell metabolic activity. (MTT. The assay for cytotoxicity).



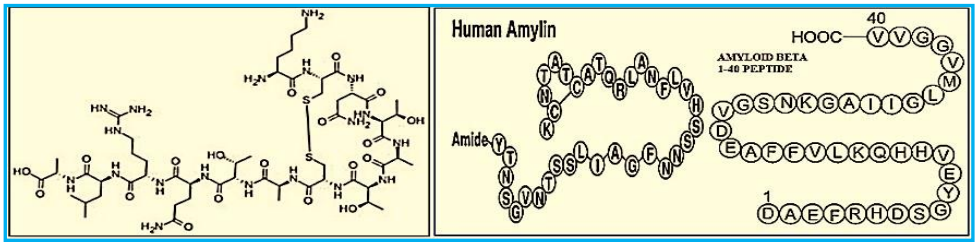
Results

Castalgin, its enantiomer vescalagin, SEN304 and inh3 show a substantial decrease in the fluorescence i.e. lower amyloid peptide aggregation - than for the peptide alone. These results have been confirmed by AFM imaging (absence of fibers with the inhibitors, while plaques of fibers were seen otherwise), while computational simulations combined with NMR allowed determining the binding sites of these molecules within the peptide structure.

Aβ Aggregation Inhibitors

The N-methylated [142] short peptide Inh3 (see above) is an effective inhibitor of Aβ aggregation. The short poly-N-methylated Inh3 is more effective than the complex polyphenolic natural products VESCALAGIN and its enantiomer Castalagin.

It was found that many biologically active agents that affect positive effects on the aggregation of neuropeptides like A β and Amylin are also antibacterial. Both Aβ and Amylin, the fibril forming polypeptides active in the brain, are antibacterial as well.



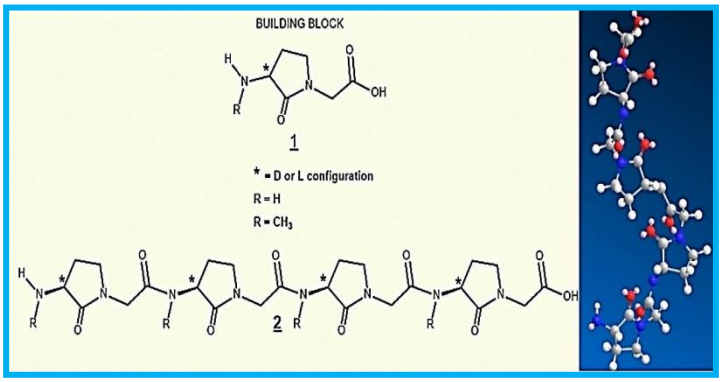
Synthetic Amylin (also antibacterial) Amylin and Aβ (forming fibrils, antibacterial)

Investigations into other natural products and synthetic peptide libraries have further highlighted the importance of cyclization and N-methylation for pharmacological properties to some of these properties that reduce flexibility and excellent proteolysis resistance, and provide the ability to penetrate biological membranes by reducing the number of hydrogen and hydrogen bonds using N- methylation [143]. Both cyclization and N-methylation support the formation of intramolecular hydrogen bonds which further decreases flexibility and solvation. Interestingly, most studies of passive membrane perfusion by cyclic peptide libraries have demonstrated that the impact of N-methylation on membrane permeability was highly positioned dependent and not necessary correlated with increasing N- methyl content [144]. We therefore turned to new alternatives, seeking at first to improve selectivity via short (truncated) analogs of natural Dermaseptins. This strategy also turned out to be disappointing although not devoid of some interest.

Thus, elimination of flexible peptide domains indeed led to short α-helical derivatives with reduced hydrophobicity but with improved selectivity [145,146].

The Challenge

The introduction of N-Me units on a short peptide based on β -turn mimics as building blocks can improve selectivity in Gram-positive vs. Gram-negative bacterial eradication. The changes in the flexibility of these agents can also be expressed in selective hindering of fibril-forming peptides (Aβ and Amylin for example). The building β-turn mimics in this work will be based on Freidinger lactams [147]:

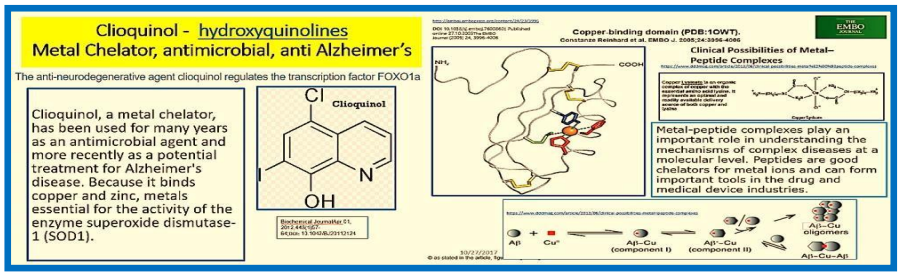


Octa-peptide surrogates based on Freidinger's lactam and MMI local minimum structure CChem 3D presentation

Foldamers are a very prominent class of α-helix mimetic peptides [148]. They consist of amino acid α / β-amino acid oligomers), or N-substitute glycine residues (peptoids). Such coefficients have been shown to inhibit the proteolytic activity of secretase, an enzyme involved in the processing of amyloid-β (Aβ) in Alzheimer's disease by blocking the initial site of the substrate- **[149].**

There is a possibility that these octapeptide surrogates will mimic an α-helix [150] which is an active form that might interrupt the formation of the self-assembly Aβ - fibril aggregates.

Antimicrobial chemokines interact with receptors to realize chemotactic functions. They share a similar fold consisting of a three-stranded sheet followed by one α-helix at the C-terminus The N-terminal region is frequently disordered [151-153].



Destroyers of Aβ Aggregates

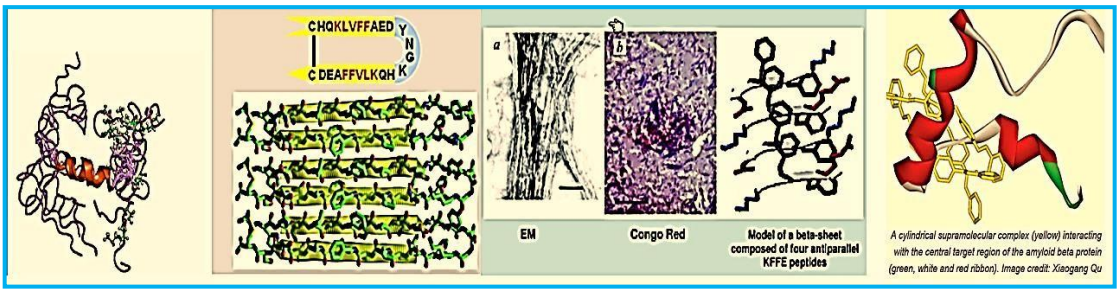
Protective properties of autophagy in neurodegenerative and infectious diseases. Contribution of antimicrobial properties to anti-neurodegenerative agents. (deters self-devouring apoptotic processes) [154]

Autophagy or "self-devour" is a natural, regulated, destructive mechanism of the cell that dis-assembles unnecessary or non-functioning ingredients. This allows for orderly degradation and recycling of cellular components. Autophagy is considered initially a nonselective bulk degradation process, data aggregation now supports the concept of selective macro-autophagic, where the cell uses receptor proteins to enhance the integration of specific charges into autophagosomes.The canonical model for this process involves these receptors binding to cargoes, typically via interaction with ubiquitinated motifs, and the receptor binding to the autophagosome membrane protein via -interacting domains. However, some classical receptors, may not require the binding to be incorporated into autophagosomes. Although systematic studies have not yet been performed, many of these receptors appear to be able to assist autophagic capture of both neurodegenerative disease-causing proteins and infectious agents. In their antimicrobial role, these receptors are referred to as a new class of pattern recognition receptors termed sequestosome.The ability of receptor proteins to recruit substrates to autophagosomes can also be modulated by posttranslational modifications. Although systematic studies have not yet been performed, many of the receptors, including p62 and optineurin, appear to be able to assist autophagic capture of both neurodegenerative disease-causing proteins and infectious agents.

Autophagy also regulates inflammation. As recently reviewed), the anti- inflammatory functions of autophagy in principle involve: Prevention of spurious inflammasome activation and down-regulation of the response once inflammasome is activated and

Inhibition Responses: The underlying processes include autophagic elimination of endogenous damage-associated molecular patterns (DAMPs) e.g., depolarized mitochondria leaking ROS, mitochondrial DNA, and oxidized mitochondrial DNA, which lowers the threshold for inflammasome activation, or direct targeting and degradation of inflammasome components and products.

This, in turn, tapers the intensity and duration of inflammasome activation. However, the engagement of autophagy with cellular outputs of a prototypical unconventionally secreted protein, is more complicated. Autophagy assists secretion of a cytosolic protein that lacks a signal peptide and is unable to enter the conventional secretory pathway via the ER and Golgi. Thus, autophagy also plays a decisive role in delivering the protein and possibly other proinflammatory substrates, once they are correctly activated in the cytosol, to the extracellular space where they perform their signaling functions.



Amyloid Fibril Formation an Inhibition based on the KFFE unit credit ref [19,155].

In this project the effect of chirality N-methylation and α -helix will be studied. Inhibition of Aβ fibril formation will be studied as well.

Similarities in Sructure and Function Antimicrobial Peptides (AMP) and Fibril-Forming Peptides

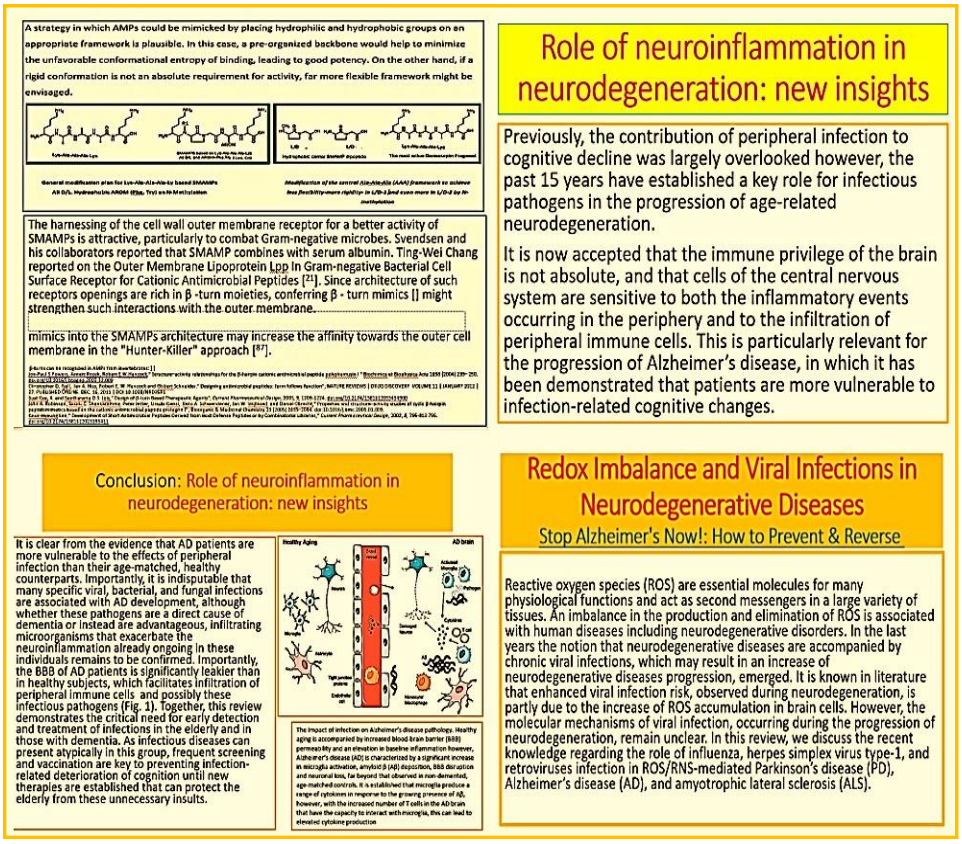
There is accumulating evidence [156-25] that differentially microbial components stimulate the transcription, by microglial cells, of two antimicrobial peptide genes, products that rapidly accumulate at CNS sites that have regenerated following axotomy. Interactions between peptides and fats are of fundamental importance in the functioning of many intracellular membrane-mediated processes including antimicrobial peptide activity, hormone receptor interactions, the bioavailability of drugs over the blood-brain barrier and viral fusion processes Brain accumulation of β-amyloid (Aβ) is considered to play a significant role in the etiology of Alzheimer's disease. Host biomolecules that target these pathogens, for example, microbial peptides (AMPs) such as Aβ itself, are an exciting option for follow-up and diagnostic tracking of such brain infections.

In this work, we demonstrate improved microbial activity (antimicrobial activity against microorganisms is measured in vitro by a minimal inhibitory concentration of peptide (MIC), defined as the lowest concentration that can inhibit overnight growth) of β-pins mimicking the host mi- natural. These peptide substitutes were based on N-methylated disease, aimed at reducing the elasticity and stabilizing the peptide's confirmation. This may eventually stimulate Aβ overproduction and aggregation. Host biomolecules that target these pathogens, for example, microbial peptides (AMPs) such as Aβ itself, are an exciting option for follow-up and diagnostic tracking of such brain infections.

Studies show that the harmful effects of Protegrin-1 (PG-1) on the gram-negative bacterial membrane are primarily dictated

by the abundance of lipid A and phosphonic lipids in the outer and internal membranes. Just as the insertion of PG-1 into lipids and the monolayers can account for its potential to expand and permeabilize the outer cell wall of E. coli, similar effects on rich phosphatidylglycerol monolayers can contribute to its ability to permeabilize the internal membrane (cytoplasmic). This inherent unique ability to "recognize" and interact with structures or structural patterns that are intrinsic to bacteria-free eukaryotic cells may well predict the development of proteins and other antimicrobial peptides as future therapeutic drugs [157].

Models of Toxic β-Sheet Channels of Protegrin-1 Suggest a Common Subunit Organization Motif Shared with Toxic Alzheimer β-Amyloid Ion Channels [158]. Damage in the Blood-Brain Barrier (BBB) can also become a source of neuroinflammation [159].



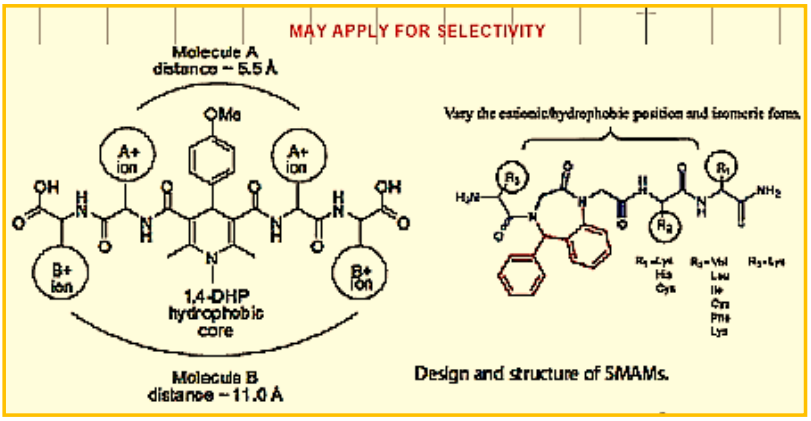
Role of Inflammation in Neurodegenerative diseases

Conformation of the peptide surrogate. Furthermore, these synthetic peptide mimics (SMAMPs). In the Protegrin 1 [160] case Showed direct interaction with β protein- protein protein Lipopolysaccharides (LptD), which determines them apart from other antimicrobial peptides, whose effect is primarily based on membrane activity [161].

It is of critical importance to neurons, which have developed comprehensive and complex pathways to pair Ca2 + signal to their biochemical machines.

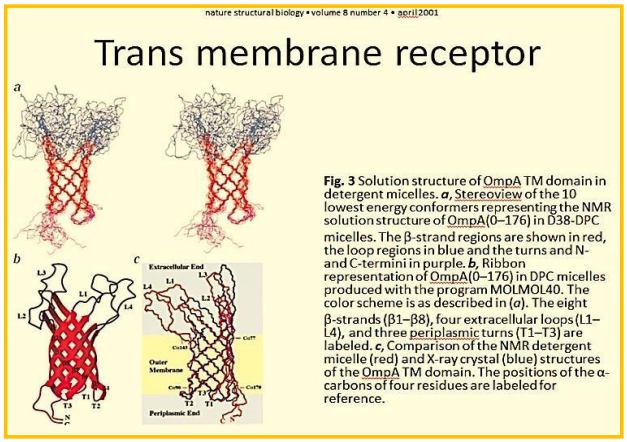
In particular, Ca2 + participates in transmitting the depolarizing signal and contributes to synaptic activity. During aging and the processes of neurodegenerative diseases, the ability of neurons to maintain an adequate level of energy may be impaired, thus affecting Ca2 + ions homeostasis.

In Parkinson's disease (PD), many symptoms of neurodegeneration develop from the mitochondrial function, which is damaged by the specific effects of toxins on the mitochondrial respiratory chain and / or genetic mutations. Although these effects are found in almost all cell types, the most prominent characteristic of PD is the extreme selectivity of cell loss, which is limited to dopaminergic neurons in the central part of the nigra pars compacta material, Escherichia coli (E.coli), Clostridium difficile, Burkholderia cepacia Klebsiella, Staphylococcus aureus, Streptococcus pyogenes, Tuberculosis Mycobacterium, Acinetobacter baumannii, microorganisms and leave these "useful" intact.



Now it is determined that small molecules based on simple motifs [162] can be prepared to eliminate Gram + as well as grammatical. The next step is the stage of differentiation (attacking only the unwanted bacteria). The selectivity state we had in mind is to establish the surrogate parasite peptide on the mimic β -turn range known to interact with cell wall proteins. In bacteria mainly the outer part of the transmembrane receptors [163]:

Outer Membrane Proteins that are Found in The Outer Membranes Of Gram-Negative Bacteria



The cell wall of the bacteria contains proteins. Especially those that build the toll cell wall, TLRn (n=1-13) thermal membrane [164] signaling [165] receptors [166-168] for example. The current study reveals that short (as only 4-5 amino acid sequences, PXXP mentioned above, for example), polypeptide chains are once forming a non-covalent connection to the receptor on the outer membrane. This region of the protein [169]. The interaction of drug antibiotics is descriscribed.

Some trans-membrane receptors-Bonding short helix in non-covalent bond [171]

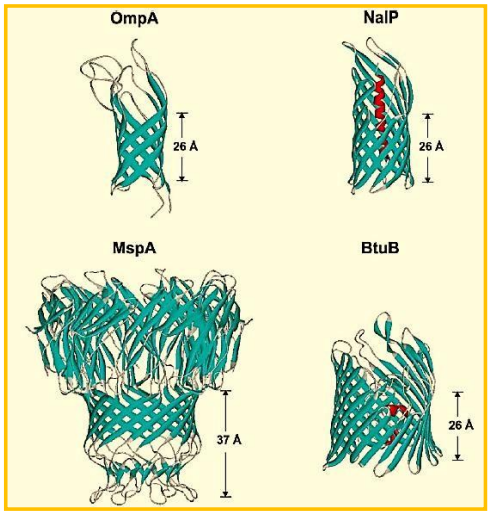
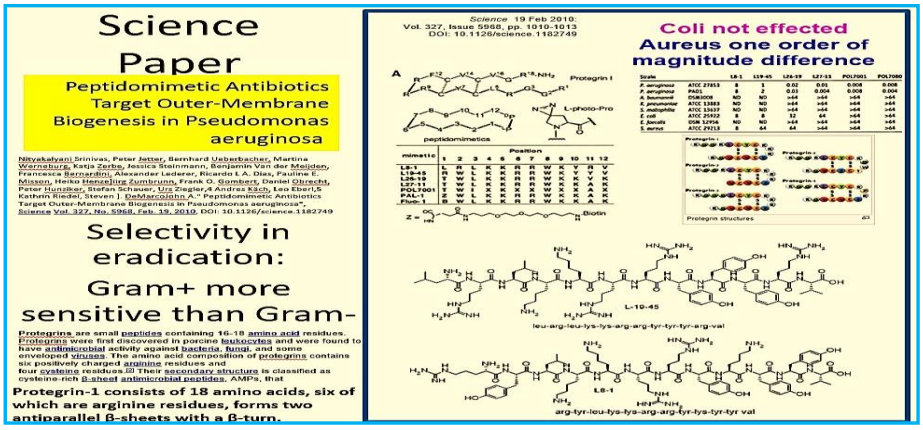


Figure: Representative structures of h-barrel membrane proteins. OmpA, the transmembrane domain of OmpA of E. coli (PDB entry 1G90; NalP, translocator domain of autotransporter of N. meningitidis (1UYN;) MspA, porin of M. smegmatis (1UUN); BtuB, cobalamin transporter of E. coli (1NQE;). The approximate location of the lipid bilayer is indicated in each structure. Note the much wider hydrophobic thickness of MspA.

From which the receptor becomes rich in motifs β -turn [172] motifs can provide land and non- covalent bonds. It may be possible that antibacterial peptide substitutes, based on imitations of such short peptide sequences, can attach to the proteomic portion of the outer domain that transient membrane receptor (LTR4 and LTR5 in particular). It is selectively made in the selection of bacteria to eliminate. The investigation will be done by the mechanism of normal bacterial cell dissolution.

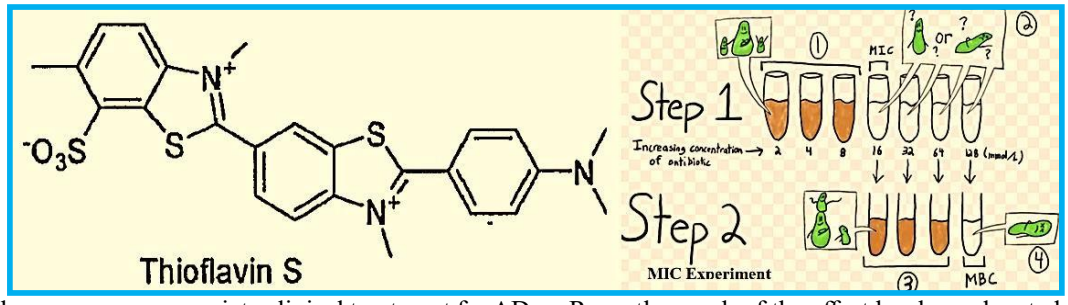
Protein-Protein Interfaces [173,174] interactions can be satisfied by adding a second copy of the interface domain to a monolithic polypeptide in such a way as to allow it to interact with the original interface (The second strategy was transferred by Mossing and Sauer [175]. When they were connected by rotation, a partial copy of the video interface of the protein and the DNA to protect the protein until the end of the whole copy. stable).



In this media we examined the synthesis and initial test for broad-band bacteria based on β-turn mimicking mimicking eradication of the hemolysis of human red blood cells.

Into The Practice Guidelines

The foldamers will be prepared in the synthesis lab based on ref. and Freidinger's work ref. [16], in both chiral forms starting from [D] and [L] methionine. Human red blood cells hemolysis (RBC) will be determined and MIC experiments carried out to determine antibacterial activity on. Coli (gram-negative) AND Staph. Aureus (gram- positive) bacteria according to ref. and references cited therein. Interaction with Aβ fibrils will be carried out according to, and determined spectroscopically using THIOFLAVEN-S as a fluorescent indicator.



There is currently no cure or appropriate clinical treatment for AD, and it is still not clear how the ad comes and circulates throughout the brain and central nervous system (CNS)." Recent studies by GWAS indicate that a significant proportion of AD- related gene markers are located in gene coding regions, suggesting the contribution of epigenetic or environmental factors to AD risk. The potential contribution of pathogenic bacteria to aging and Alzheimer's disease is increasing [176].

Hopes

We hope to find antibacterial activity (preferred Gram Selective) and destruction of Aβ Fibrils [177].

Recently, much of the effort has been devoted to the development of GS analog with an improved therapeutic index, in which the antimicrobial and cytotoxic activity (eg, the quality) has been severed. In this journey, both β-strand and β-turn have regions are widely modified in SAR studies that shed light on the factors that govern bioactivities, such as cationic nature, amphipathic nature, β sheet structure, and global hydrophobic ring size [178]. The breakthrough of AMPs into the membrane was a simulated computer. High-resolution structures and orientations of antimicrobial peptides Piscidin 1 and Piscidin 3 in fluid bilayers reveal bias, kink, and Bilayer immersion [179]. Furthermore, the bacterial outer lipoprotein Lpp membrane does gram-negative bacterial cell surface receptors for cationic antimicrobial peptides

[180]. The outer membrane protein Lpp of Gram-negative Gram acts as a receptor for an antibacterial peptide. Scientists identify and characterize the Lpp, which is responsible for the recognition of the antimicrobial action peptide. Lpp is a new target of an antimicrobial peptide. The app may be used to the ligand to develop microbial materials.

Antimicrobial peptide surrogates seem to pave a promising route to agents that will become useful in the combat with the nosocomial infections pandemic. Many targets might be achieved for example: Novel antiseptic agents or disinfection with advanced chemicals based on active motifs identified in antimicrobial peptides.

Although many mechanisms of action are possible for AMPs and their surrogates, the facts of minor toxicity, low re4sistance and simple access to the surrogates turn the once elusive targets achievable.

Although antimicrobial polymers attract considerable attention, There are 6 significant efforts [181-186] with "small" molecules in this regard FIG 1:

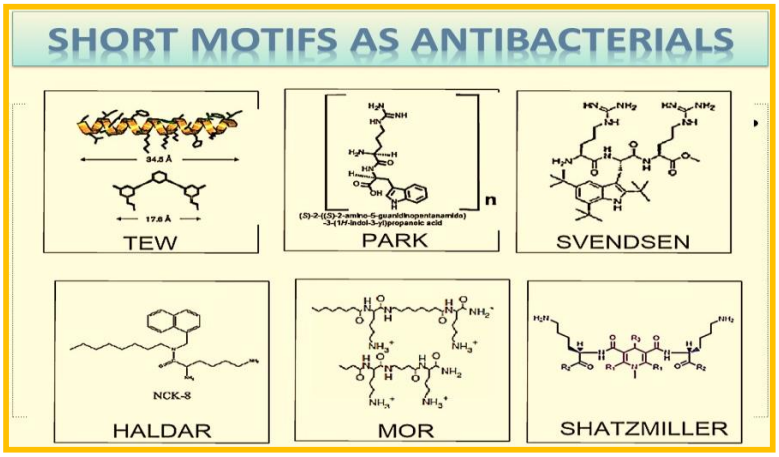
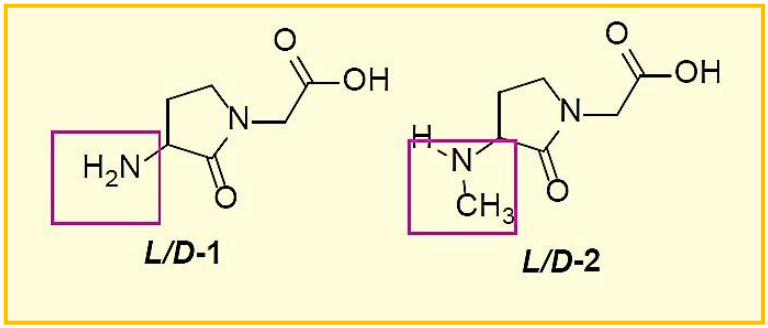


Figure: Surrogates of biological potent motifs of antimicrobial peptides.

The commercial development of one such surrogate is currently in progress expanding the horizon of AMP surrogate to the area of cancer by the company LYTIX [187]. The compound is characterized as amphipathic in which the central hydrophobic moiety is flanked by cationic amino acids. Lysin and/or Arginine are usually applied (FIG 1).

Usual eradication occurs at submicromolar concentrations where E. Coli (Gram- negative bacteria) is eradicated at half of the biocide concentrations than the S,Aurous (Gram-positive bacteria). The short peptide L-lysyl-L-alanyl-L-alanyl-L- alanyl-L-lysine was identified as a capable bacteria eradicating motif.



Freidinger's lactams and N-methylated analog.

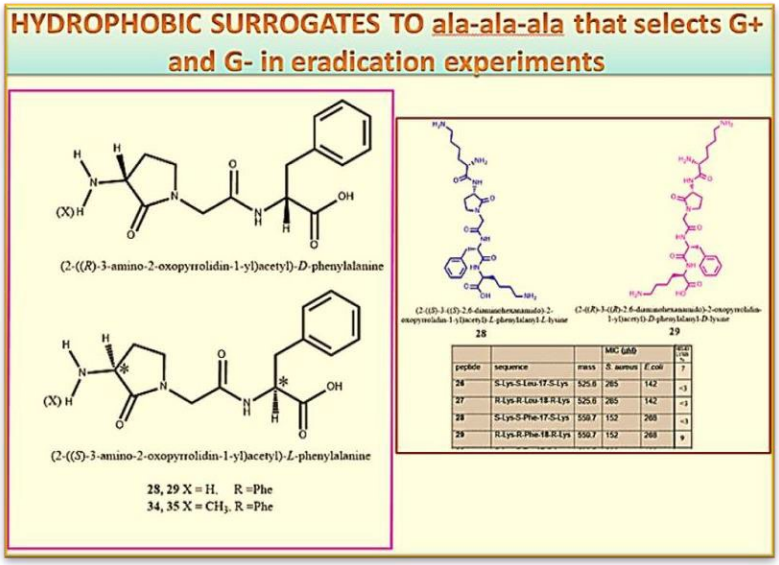


Figure: Unusual order of bioactivity. β -turn-based surrogates eradicate better the gram-positive bacteria.

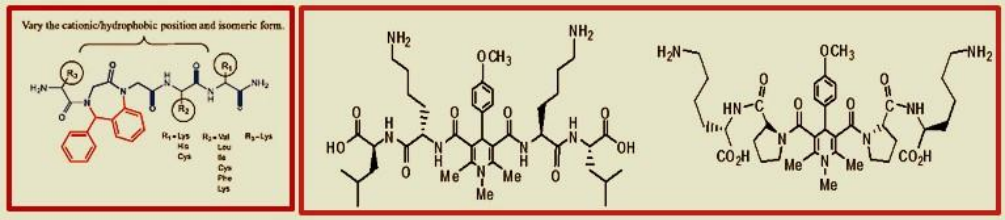
However, the application of β -turn mimic (Freidinger lactam) in these cases, the pair of enantiomers, the AMP s surrogates 28 and 29 (FIG. 2) proved more active towards Gram-positive bacteria than towards theGram-negative bacteria. In these cases, a remarkable selectivity was obtained by a structural change in the eradicating molecule.

It is not clear in what mechanism this eradication occurs. Today, many different routes have been identified for the eradication of bacteria from Carpeting to DNA damage [188]. Selection of bacteria from the vast variety surrounding our life on earth and living in symbiosis in our bodies is an obstacle that has to be removed in case antibacterial agents are going to be applied by humans. For this, a bacteria-selective variants are needed for further exploitation of AMPs as potential antibacterial agents [189].

The Synthesis of Short Antibacterial Peptide Surrogates Centered on a β-turn Mimic

There is a pressing demand for new antibiotics which are effective against drug- resistant bacteria without contributing to resistance development [190].Due to their unique mechanism of action, Anti-Microbial Peptides, the AMPs, (the carpet mechanism), have shown no bacterial, fungal and viral Resistance [191]. We have designed and developed antimicrobial short peptide surrogates that include β -turn We designed and developed short microbial peptides that include factors of β -turn, and [two] lysines that rely on their sequences and with cationic amphibious structures based on imitation of microbial peptides naturally occurring at deficient concentrations, . These short peptide peptides exhibit this vigorous antimicrobial activity against a wide range of bacteria including E. coli methicillin-resistant Staphylococcus aureus without harmful hemolytic activity. It should be noted that these short peptide substitutes did not lead to the development of resistance to E. coli measurement. The MIC experiments indicate that the peptide solutions of the D- based and L-are antimicrobial (CAMP) peptides are almost identical in both Gram and Gram bacteria. These results indicate similar behavior of artificial “and” natural “and” natural “L substitutes when they bind the bacterial membranes. There is, however, sensitivity to chirality in RMC hemolysis.We may contribute to further understanding of how CAMPs sense microbial membrane as well as provide a new direction for developing new disruptive membrane agents [192]. The peptide- surrogate design principle offers significant flexibility and diversity in the creation of new antimicrobial materials and their potential biomedical applications [193].

We have published on the synthesis and evaluation of antibacterial compounds based on am amphipathic motif, observed as a short peptide unit, namely **L-AAA-L**, found in many antimicrobial natural peptides, of a five-amino-acid linear chain where the central hydrophobic 3 amino acids unit is flanked by two lysine units and prepared some surrogates of these Penta- **peptide K-A1A2A3-K** segment including compounds where privileged scaffolds are introduced, substituting a part of the hydrophobic amino-acids part structure.



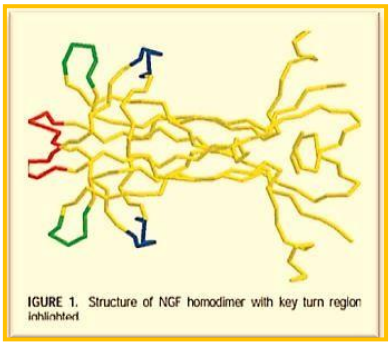
K-A^{1}A^{2}A^3-K based surrogates.

The peptide surrogate 2 shows a structure where β -turn mimic (benzodiazepine unit)[194] is conferred to A1 in the drawing above. In this paper we report on conferring other β -turn mimics as a potential center of selective attachment to bacterial cell walls.

Introduction

The recognition of protein-protein, often observed in the interactions of antibodies with proteins in living organisms, is one of the main phenomena still understood and exploited by drug researchers. Peptides and proteins are essential to many life- supporting processes. The interaction between the peptide ligands and their receptor targets usually involves β structures to evacuate. However, poor bioavailability and bad pharmacokinetics significantly impairs the use of peptides as drugs. Low molecular weight imaging agents direct prostate-specific prostate antigen (PSMA). PSMA is a separate type II membrane protein with an abundant and limited expression on the surface of prostate cancer (PCa), especially in prostate cancer [195].

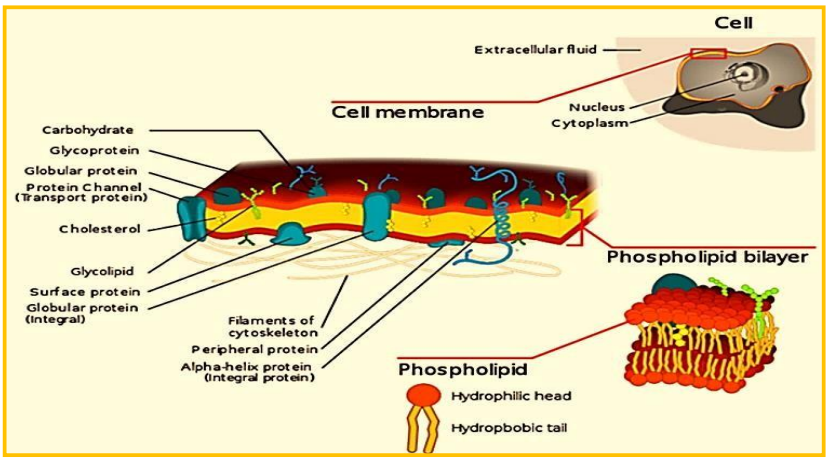
Protein-protein interactions (PPIs) regulate a wide array of cellular processes and are attractive targets for drug design. β -turn mimics can interact and bring about recognition and association of proteins. It was also noted that some of the abilities of AMPs to combine with cell walls of microbes. The possible role of a PXXP central hinge in the antibacterial activity and Membrane Interaction of PMAP-23, a member of the cathelicidin family. This is due to the short peptide motifs PXXXP [196]. This might contribute to Catherin endocytosis. Regarding endocytosis, the structural and chemical requirement were investigated and Sequence YXRF Implicates a tight turn as the structural recognition motif for endocytosis [197,198]. Investigators examined the use of β -turn mimics in the protein-protein interactions involving Transmembrane receptors in nerve cells in protein interactions that involve β turns [199]. Such β -Turn mimics can either mimic or disrupt Protein-Protein Interactions (Figure 1below).



IGURE 1. Structure of NGF homodimer with key turn region inhlighted

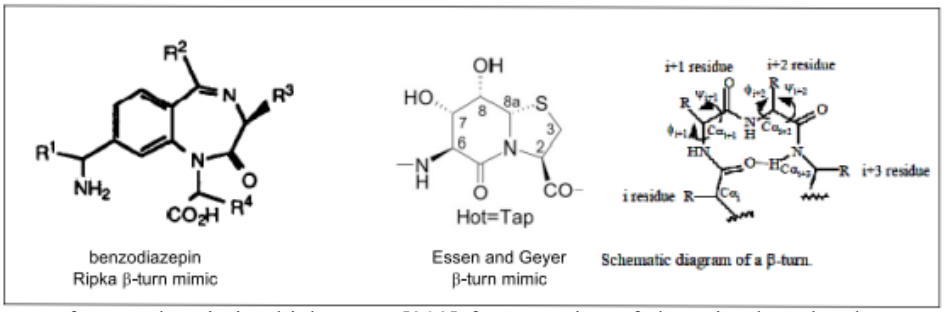
Almost half the mass of the outer membrane of the bacterium is protein. Most “outer membrane” proteins are thought to be located only in the outer membrane, although some proteins are present in both the outer and cytoplasmic membranes. Bacteria produce cell walls with the exception of mycoplasmas, and the cell wall component common to all eubacteria is the murein, or the peptidoglycan, which contributes mechanical rigidity. All gram-negative bacteria contain an additional layer in the cell wall structure, i.e., the outer membrane, which is on the outside the peptidoglycan layer and shows up as a tri-laminar architecture on the electron micrographs of thin sections of these bacteria [200].

One of the approaches is to learn about the potential application of short peptide mimics like β -turn mimics, on the recognition with perspective to apply this if future drug design. The appearance of β -turns in protein interaction is by far more common than that of other, like β -turns. Noncovalent [201] interactions between the turn-mimics and some receptors on the cell wall of bacteria may supply enough energy differences that may allow differentiation between various bacterial transmembranal cell wall receptors due to receptor- β -turn mimic interactions. The interactions of some β -turn mimic with many classes of proteins which vary in their seconddary structure (β -sheets, globular) has been found to rely on the interaction between β -turn mimics and the proteins.



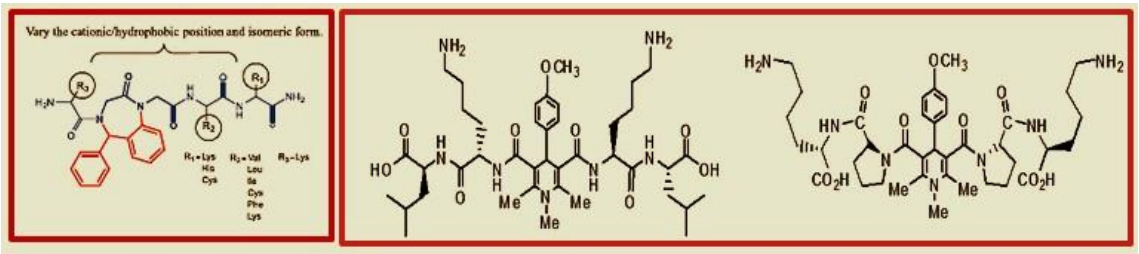
Results and Discussion

Many variants of β -turn mimics have been applied so far in this area of research. One can read about benzodiazepines, β-turn mimic Hot═Tap for example [202].



There is a growing urge for novel antimicrobial agents [203] for therapy but also for Hygiene and Agriculture, Soil Sterilization, for example. The class of compounds in focus is the growing group of polypeptides isolates as part of the host defense systems of all living on earth (Antimicrobial peptides).

Strains of the microbes that harm are becoming more resistant to drugs but also live in the vicinity, in the same organism, like other useful and needed fauna of microorganism to exist in the human gut, the "beneficial" various strands of Firmicutes, Bacteroidetes,Actinobacteria, and Proteobacteria for example. We would like to selectively kill the "bad" microorganisms and leave the "useful" ones intact.



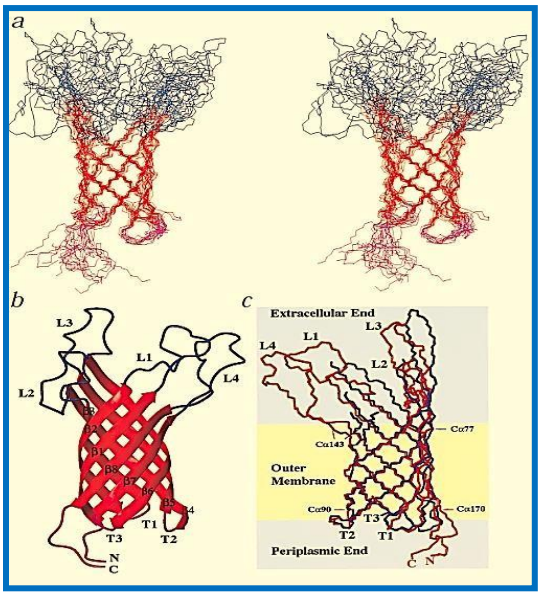
K-A1A2A3-K Based Surrogates of pentapeptide antibacterial motif K-A1A2A3-K.

Now it is established that simple small molecules based on simple motifs [204] can be prepared and eradicate Gram+ as well as Gram- bacteria. The next step is the differentiation stage (to attack only the unwanted bacteria). The selectivity mode we had in mind is to base the eradicating peptide surrogate on β-turn mimics that are known to interact with cell wall proteins [205]. The presence of additional β-sheets in these proteins is discussed, but it may not be expected that the membrane is being coped by a mixture of α-helices and β -sheets that the main hydrogen chain bond contributors and acceptors on the sheet edges cannot be supplemented by those of Coils. This amide *saturation* problem at the end of the wires of the transmembrane h-sheet can be eliminated, however, if both ends associate to form a barrel.

Exact membrane protein to come. Two types, α-helical proteins and α- barrel. In both types, all donor bonding and hydrogen acceptors of the polypeptide backbone are completely compensated and buried while the non-polar side chains point to the membrane. The α-helical type is more abundant, and it occurs in cytoplasmic (or internal) membranes, whereas the marine life is known from external membranes of bacteria. The construction of the barrel is

described by the number of wires and the sheer number, which is a measure of the inclination angle of the wires on the barrel axis. The right hand and left-hand twist require a slightly larger shear number than several strands. Membrane protein-barrels contain between 8 and 22 h-strands and have a simple topology that is probably enforced by the folding process. The smallest nests create reverse emails and work as enzymes or bind to other macromolecules. A medium barrel term is a more or less specific porosity for nutrient uptake, whereas primary barrels occur in Fe2 + active transporters. The barrel is objected suitable for channel engineering, because the structures are simple, and since many of these proteins can be manufactured for recycling bodies and returned from them.

In bacteria mainly the outer part of transmembrane receptors [206]:



Structure of outer membrane protein A transmembrane domain.

The cell wall of bacteria contains proteins [207]. Mainly those that build the cell wall Toll, TLRn (n=1-13) transmembrane signaling [208] Receptors [209-211] for instance. Current research reveals that short (as little as 4-5 amino acid sequences, PXXP that was mentioned above [1], for instance), polypeptide chains are the once forming a non-covalent attachment to the receptor on the outer membranes. This region of the protein in which the receptor is rich in β -turn motifs, could provide a ground for non-covalent connections. It may become feasible that antibacterial peptide surrogates, based on mimics of such short peptide sequences, may attach to the proteomic part of the outer domain that the transmembrane receptor.

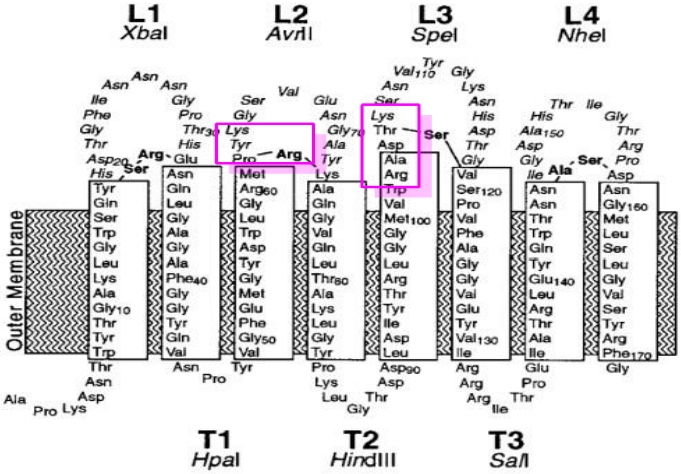


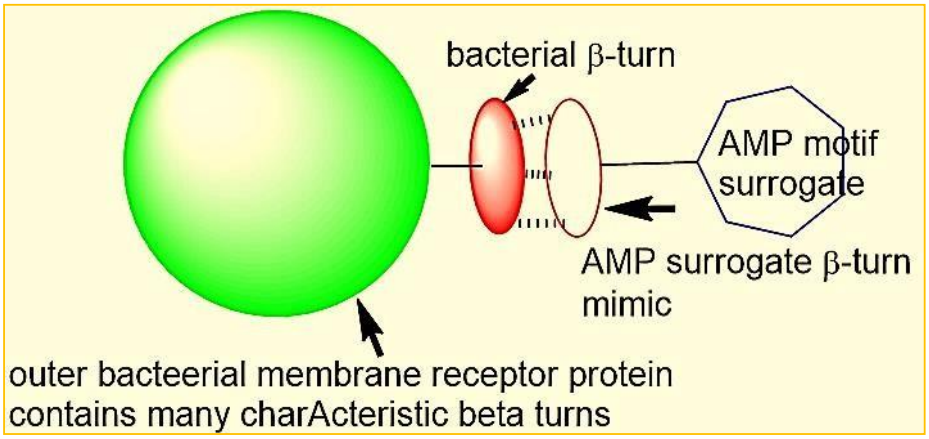
FIG. 1. Two-dimensional model of the arrangement of the N-terminal β-barrel domain of OmpA in the outer membrane, based on the prediction by Vogel

Structure K (R)-A-A-A-A-A in the Transmembrane Exterior

(LTR4 and LTR5 in particular). This is made in this way selectively in the selection of bacteria to eliminate. The elimination will be done by the normal membrane dissociation mechanism of the bacterial cell.

Results and Discussion

In this media we tested the synthesis and initial test for broadband bacteria based on β-turn mimicking the eradication of the hemolysis of human red blood cells.

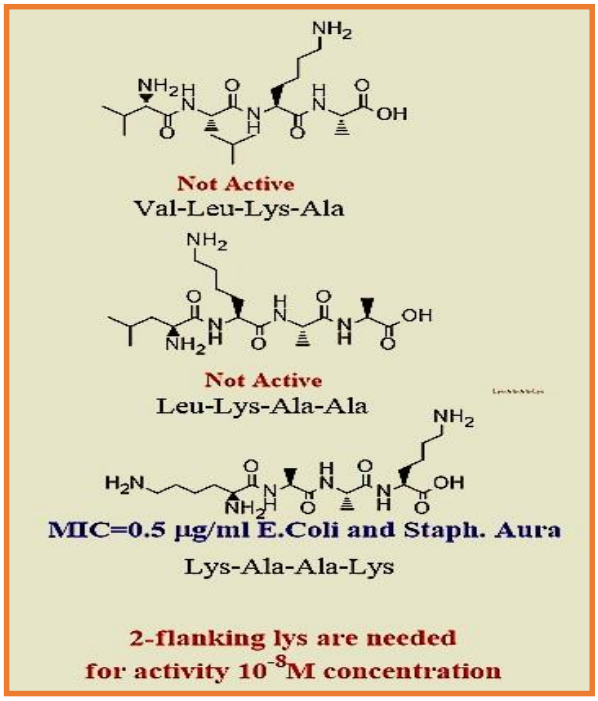


Presentation of possible approach between the bacterial outer domain of the transmembrane receptor and β-turn moiety of antimicrobial peptide surrogate.

Hereby we report of the synthesis of short antibacterial peptide surrogates and the checking of their biological activities as antibacterial substances.

In previous papers we have reported the application of benzodiazepines to mimic mimic-dihydro-pyridine scaffolds and retardation in the synthesis of a series of active spectrum-efficient antibacterial substances. Some were relative to natural AMP, antimicrobial peptide [212], typically MIC values [213] of approximately 40, ie 5-10 times more active (MIC values 12). The introduction of their imitations did not alter the normal behavior of AMP substitutes.

Focusing on a fragment of 1 amino acids from the lysine-rich part of Dermaseptin S4 [214], *Val-Leu-Lys-Ala-Ala-Ala-Lys*. We have prepared the following linear tetrapeptide linear epitopes and found that 2 lysins in flanking positions are essential for enhanced biocide activity as shown below:



3 aa and 4aa-peptides as linear epitopes.

We have further expanded our peptide-mimetic approach to take full advantage of the impact of "snorkel" [215]

Is characterized by peptides controlling the interaction of the biological structure with the cell Membranes [216]"snorkeling" is one where peptides have a long "interval" in cationic remains (lysine and arginine [217]), which can reach the lipid-water interface.

Therefore, we focused on the amphipathic amino acid motif 4-5 where three (or two) hydrophobic amino acids (free) or their surrogate is surrounded by two amino acids lysine (K) or arginine (R) (K-AA-K, K) -AAA-K), as examples (positive charge).

They are found in frog skins, (see above) [218,219] human lactoferrins [220]. Lactoferricin and human saliva which are some of the most studied AMP derived from milk protein.

Table 1 Amino Acid Sequence, Charge at pH 7 and Molecular Mass for Synthetic Lactoferricins from Different Species

Species	Sequence	J. Peptide Sci. 5: 32-45 (1999)	Name	Charge at pH 7	Molecular mass measured (calculated)
Human		TKCFQWQRNMRKVRGPPVSCI KRDS	FIR[18-42]	5.65	3163.4[a] (3163.8)
Bovine		FKCRRWQWRMKKLGAPSI TCVRRAF	LFB(17-41)	7.84	3267.2[b] (3268.2)
Murine		EKCLRWQN EMRKVGGPPLSCVKKSS	LFM(17-41)	3.65	3003.2[b] (3003.7)
Caprine		SKCYQWQR RMRKLGAPSI TCVRRTS	LFC(17-41)	6.65	3154.4[a] (3154.8)
Bovine	PEWF	KCRRWQWRMKKLGA	LFB(14-31)	6.85	2478 (2477.8)[a]
Bovine		FKCRRWQWRMKKLGA	LFB(17-31)	5.88	2066 (2065.8)[a]
Bovine		KCRRWQWRMKKLGA	LFB(18-31)	5.88	1916 (1917.6)[a]
Bovine		CRRWQWRMKKLGA	LFB(19-31)	4.88	1791 (1789.4)[a]
Bovine		RRWQWRMKKLGA	LFB(20-31)	4.91	1616 (1615.1)[a]
Bovine		KKCRRWQWRMKKLGA	LFB(17-31)K17	6.87	2048 (2045.8)[a]
Bovine		FKCFRWQWRMKKLGA	LFB(17-31)F20	5.87	2059 (2055.8)[a]
Bovine		KKCFRWQWRMKKLGA	LFB(17-31)K17,F20	4.88	2039 (2036.8)[a]
Secondary structure		←----Helix----→ <Turn> ←----Sheet----→	MOTIF IN LACTOFERRIN		R-M-K-K and/or RWQWR

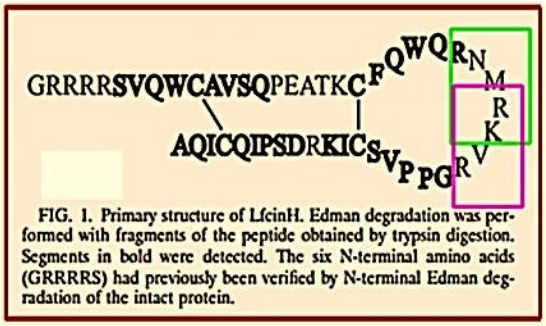
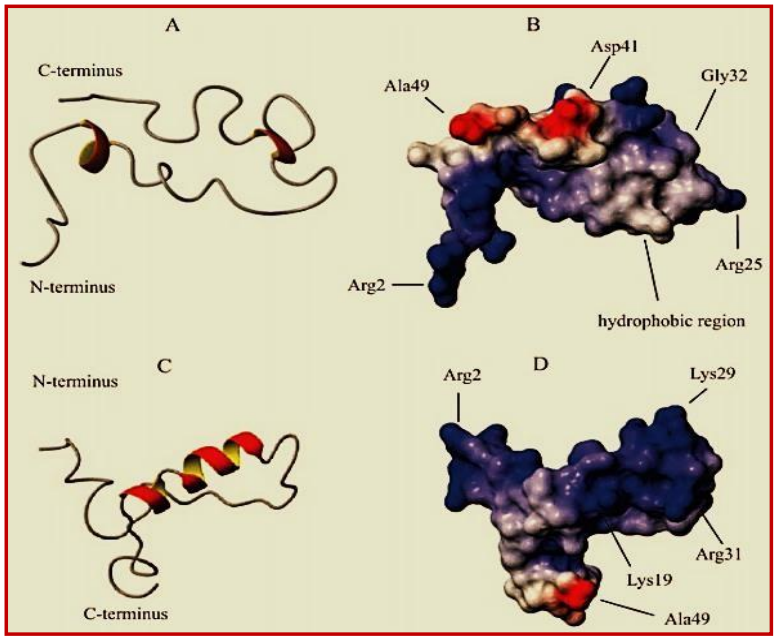


FIG. 1. Primary structure of LfcinH. Edman degradation was performed with fragments of the peptide obtained by trypsin digestion. Segments in bold were detected. The six N-terminal amino acids (GRRRRS) had previously been verified by N-terminal Edman degradation of the intact protein.

A complete sequence of lactoferricin corresponds to a 17-41 lactoferrin segment (FKCRRWQWRMKKLGAPSITCVRRAF) and sequences from this segment are also antimicrobial. Svendsen and Fogel and their groups shed light on the 3D structure of Lactoferricin [221,222].



Presentations of calculated structures of LfcinH in aqueous solvents (A and B) solvent solvent-solvent (C and D). (A and C) strip diagram representations; (B and D) distributions charge on the surface of the peptides. The positive, negative and neutral potentials are painted in blue, red and white, respectively. This figure was produced by the mogul Plan.

Protein-Protein Interfaces [223,224] interactions can be satisfied by adding a second copy of the interface domain to the monomeric polypeptide in a fashion to allow it to interact with the original interface. (The latter strategy was employed by researchers [225], when they connected via a β- turn, a partial copy of the - ribbon interface of b-crop - DNA protecting protein - to the end of an intact copy. This allowed the second copy to turn back and interacted with the remainder of the protein to form a stable monomer.)

Generally, the design [226] and synthesis of peptidomimetics are most important because of the dominant position peptide and protein-protein interactions play roles in molecular recognition and signaling, mainly living systems. The design of polypeptide mimetics can be viewed from several different perspectives [227] and peptidomimetics can be categorized in a number of different ways [228]. Examination of the comprehensive literature would suggest that medicinal and organic chemists, who deal with peptide mimics, utilize these techniques in many different ways.

Here we would like to conclude that the bend around Lys29-Arg31 (graphic D figure 2) might accommodate the antibacterial motif of lactoferricin. (chart 1 below).

Synthesis of Surrogates based on Tetra-Peptides:

Focusing on a fragment of 7 amino acids from the lysine rich part of Dermaseptin S4 [229], Val-Leu-Lys- Ala-Ala-Ala-Lys. We have prepared the following linear tetra- peptide linear epitopes and found that 2 lysins in flanking positions are needed for enhanced biocide activity as shown below:

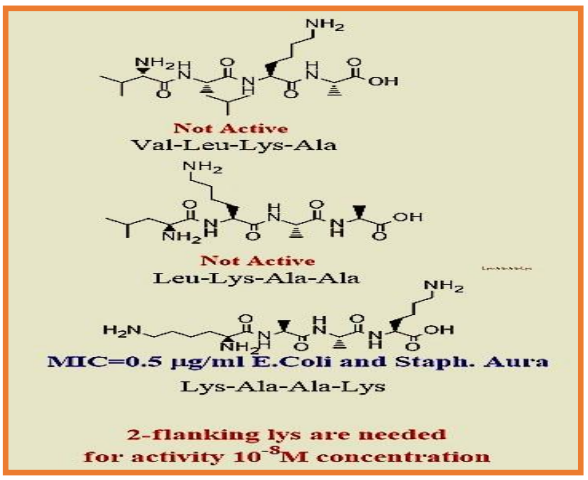


Figure: Tri and tetra-peptides as linear peptide epitopes derived from Dermaserptin S4 fragment.

Katchalski and his group in Rehovot reported in their work on Antibacterial Activity of Amino Acid Copolymers Related to Gramicidin S, on a very effective eradication of both Gram-negative and Gram- positive bacteria by short (2-5 amino acids) peptide sequences (MIC 2.5-10 µg/ml) [230]. However, The Rehovot group applied Ornithine as necessary amino acid instead of Lysine. The lysine-rich co- polymeric sequences are present in frog skins, (see above) [231,232], in human lactoferrin [233]. Lactoferricin and human saliva which is among the most studied AMP derived from the milk protein. There was no eradication when polypeptides without ornithine were tested.

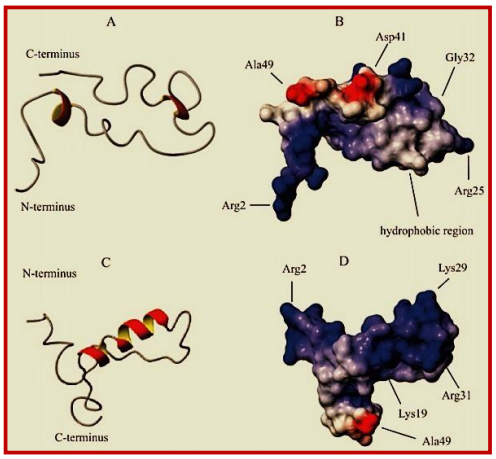


Figure 2: Representations of calculated structures of LfcinH in aqueous solvent (A and B) and membrane mimetic solvent (C and D). (A and C) Ribbon diagram representations; (B and D) charge distributions on the surface of the peptides. Positive, negative, and neutral potentials are colored blue, red and white, respectively. This figure was produced by the MOLMOL program. [234,235].

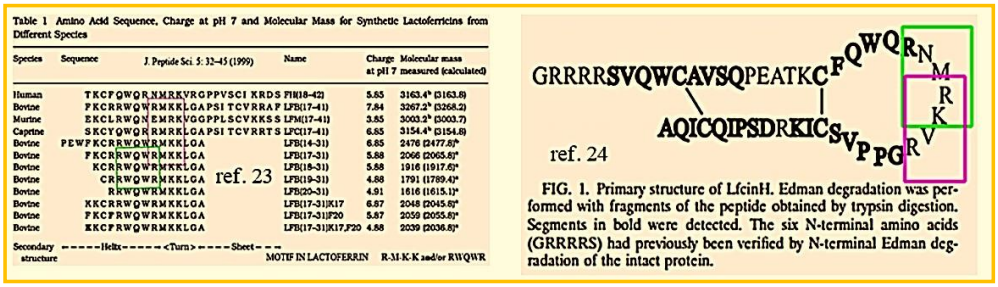


Chart 1: Lactoferricin sequences from various families of living creatures and the position of the cationic sites in the turn of the molecule (ref [42] and [43]).

In general, the design [236] and synthesis of peptide-mimetic are the most important because of peptide dominant protein-protein interactions and play molecular recognition and signaling, especially in animal systems. The design of the mimetic peptide can be seen from several different points of view [237,238] and peptide-mimetic can be classified in several different ways [239]. An examination of the vast literature will suggest that medicinal and organic chemists who mimic peptides will use these methods in many different ways.

Here we would like to suggest that the bend around Lys29-Arg31 (graphic D figure 2) might accommodate the antibacterial motif of lactoferricin (chart 1 below).

In Svendsen's work on Lactoferricin, isolated from different sources (Chart 1 below), one can identify the motifs **R-MK-K** or/ and **R-WQW-Ras** a part of the turn (Figure 2, D) in the molecule where in secondary structure assessment a turn exists. Biological activity of short peptides can be enhanced by stabilizing such turns through cyclization and /or incorporation of heterocyclic or organic constraints.

Resulting 'turn mimetics' are designed either to preserve turn-defining dihedral angles Φ and Ψ angles [240] in peptide components or to replace them altogether [241]. This may expose the active motif from the CAMP lactoferricin to the microbial outer membrane: Such small synthetic molecules that mimic surface epitopes on proteins are a potential source of novel ligands [242].

Recently, lysine was also applied as Cationic Spacer Arm Design Strategy for Control of Antimicrobial Activity in antimicrobial copolymers [243,a-c].Compounds containing quaternary ammonium cations (QUATS) are well-known antimicrobial and disinfectant agents [42c].

In our surrogate design, trying to mimic elements of secondary structures in CAMPs, primary ammonium groups (the □-NH2 groups of the lysine) serve as the source of cationic charge. We initially selected these primary amines in order to mimic the structural features of the host defense peptides, which typically contain multiple lysine residues [244] that flank the hydrophobic amino acids in the peptide. The cationic ammonium groups of peptide side chains are expected to bind to the highly negatively charged bacterial cell surface, which provides a high-affinity mechanism for the poly peptides to exert their antibacterial effects (see figure 3 below). This also facilitates the selective electrostatic attraction to bacteria cells over human cells, which have a significantly lower net negative charge on the extracellular surface.

Recent research in the area of Tuberculosis applied library of CAMPs. The tetra and penta-peptide moieties are present in the most active peptides. An example (KWIK unit) of a bioactive peptide in this [17] research:

Table 3: Antimicrobial activity of selected peptide from a library [17]. Many peptides that eradicate all sorts of bacteria (including TB) have been prepared. Thoseborn in their sequences tow positively charged amino acids that flank a hydrophobic 2 or 3 amino acids central unit were the best of them all [245].

Antimicrobial activity of selected peptides (library PL–D)						
Sequence	MIC (μM)[a]					
	M. tuberculosis	*M. smegmatis*	*P. aeruginosa*	*E. coli*	*S. enterica* serovar Typhimurium	*C. albicans*
WKWLKKWIK	1.1	1.9	2.9	5.8	11.5	5.8

Hemolysis of Human Red Blood Cells

Hemolysis of Human Red Blood Cells (RBC) reveals that the all (S) tetrapeptide (Figure 1 above) is very different from the others. It affects hemolysis to the extent of 14% whereas in all other cases throughout this paper only very poor , up to 3% hRBC hemolysis, was obtained, although the eradication was very efficient (MIC) [53] in the concentration range of 50-0.5 □M/ml.

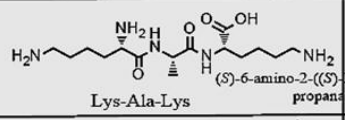
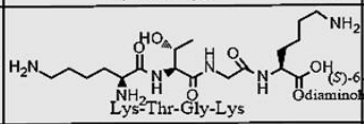
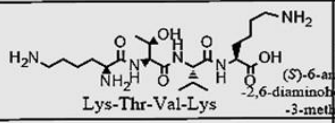
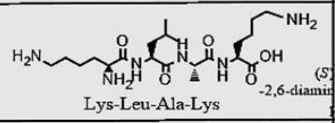
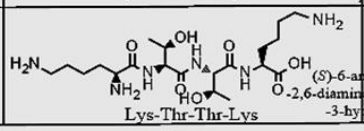
Early studies using all D-enantiomers of native and model peptides demonstrated similar antimicrobial activities of D- and

L-isoforms. Thus, the prevailing dogma supported a non-receptor type interaction for antimicrobial peptides with most pathogen membranes [246] . Since then, several studies suggest there may be notable exceptions to this generalization [247]. However, a number of studies have now shown non-equivalent activities for native all-L peptides, versus their all-D enantiomers [248] For example, in intriguing studies using PR-39, a proline- and arginine-rich peptide of porcine origin, the all- D enantiomer showed 1000-fold differences in species-specific activity against bacterial organisms [249]. These studies suggest receptor-type interactions may be meaningful for some peptides in targeting specific epitopes on the microbial surface.However, AMPs are practically cell-selective, and a high number of studies focused on the improvement of cell selectivity of AMPs were in the right direction. The results suggest that vigorous antimicrobial activity and less cytotoxicity can be achieved by increasing the net positive charge of the peptide with minimal hydrophobicity above a threshold. This is consistent with the hypothesis that the lipid composition of cell surfaces primarily determines cell selectivity. The hydrophobicity effectively responsible for cytotoxicity is that on the hydrophobic face of the amphipathic secondary structure formed upon binding to the membrane. Residues close to the ends of a helix do not sufficiently contribute to the sufficient hydrophobicity [250].

With this in mind, we have prepared a series of tetra-peptides in which two lysine rests flank 2 hydrophobic units. The MIC [251] results show that all are eradicating bacteria in 10-8M conc.

Table 4: Structures and bactericidal activity (MIC) of retro-enantio tetra peptides.

Structures and bactericide activity of tetrapeptides, MIC and hRBC

cpd	structure	MIC Coli / Aur	hRBC %
1	 Lys-Ala-Lys	not active	14
	(*S*)-6-amino-2-((*S*)-2-((*S*)-2,6-diaminohexanamido) propanamido)hexanoic acid		
2	 Lys-Thr-Gly-Lys	0.5μg/ml 10^{-7} - 10^{-8} M	-<3
	(*S*)-6-amino-2-(2-((2*S*,3*R*)-2-((*S*)-2,6 diaminohexanamido)-3-hydroxybutanamido) acetamido)hexanoic acid		
3	 Lys-Thr-Val-Lys	0.5μg/ml 10^{-7} - 10^{-8} M	<3
	(*S*)-6-amino-2-((*S*)-2-((2*S*,3*R*)-2-((*S*) -2,6-diaminohexanamido)-3-hydroxybutanamido) -3-methylbutanamido)hexanoic acid		
4	 Lys-Leu-Ala-Lys	0.5μg/ml 10^{-7} - 10^{-8} M	>8
	(*S*)-6-amino-2-((*S*)-2-((*S*)-2-((*S*) -2,6-diaminohexanamido)-4-methylpentanamido) propanamido)hexanoic acid		
5	 Lys-Thr-Thr-Lys	0.5μg/ml 10^{-7} - 10^{-8} M	<3
	(*S*)-6-amino-2-((2*S*,3*R*)-2-((2*S*,3*R*)-2-((*S*) -2,6-diaminohexanamido)-3-hydroxybutanamido) -3-hydroxybutanamido)hexanoic acid		

We have synthesized and tested (MIC) tri-, tetra- and the above-mentioned penta- peptides in which the central part of the amphipathic compounds consists of hydrophobic amino acids and this is flanked by two lysine units as mimics of the natural situation.

In our hands, tri-peptides did show poor biocide activity. However, the tetra-peptides shown below were very potent in the eradication of both Gram+ (Staph. Auer.) as well as Gram- (E. Coli) bacteria in 10-7-10-9M/ml concentrations. Similar to the natural CAMPs, only little (>5-3%) hRBC hemolysis is obtained on incubation of hRBCs (from healthy human blood).

The in the above table tetra-peptides are of "unnatural" configuration. The results indicate the following main features:

1. Cell mortality is caused by the 2 flanking Lysine units that are connected to hydrophilic dipeptides constructed of 2 "unnatural" (R) - configured hydrophobic amino acids in the amphipathic short peptide.

2. The spacial arrangements of the structures of these units are not relevant as for the bacteria eradication process. Presumably, the spatial arrangement of bacterial receptors does not dictate any event in the course of action of the eradicating peptide. The mortality of these bacteria strands most likely takes its course by the disintegration of the cell membrane.

It seems that the cationic ends of the biocide peptides are causing the membrane disruption. Katchalski, Volcani and collaborators reported the biocide activity of poly-flank. Unfortunately also causing blood cells agglutination [252]. Biocidal co-polymers have cationic amino acids like lysine to affect eradication of microbes.

Why Gram-Positive Bacteria are Easier to Eradicate with the N-CH3 Analogs?

Nosocomial infection is the leading cause of death and increased

morbidity among inpatients throughout the world. From data conducted by the World Health Organization (WHO), it was found that 17 of every 100 hospitalized patients would present with hospital-acquired infections in both developed and developing countries at any given time. Nosocomial infections are already in the frame of a pandemic [253]. Staphylococcus aureus and enterococci are the most commonly isolated bacteria causing nosocomial infections. Among those giving therapeutic problems are methicillin-resistant staphylococci and vancomycin-resistant enterococci. The need for active novel agents for combat is urgent [254]. The problem is in the stage of corrupting the environment, first nearby to healthcare facilities [255]. Antimicrobials, focusing on short antimicrobial peptide surrogates,(The use of peptidomimetics 256 allows one to mimic the natural structure by introducing non-natural amino acids [257]) or SAMPS, are a promising [258] outlet from this grim situation. In this communication we present a finding that may lead the chemical contribution to the combat: Preference of eradication of gram-positive microbes caused by a structural alternation: N-methylation. The different cell envelopes architecture of the two sorts of bacteria permit the N-methylation [259], inducing conformational changes to alter the preference toward the gram-positive bacteria.

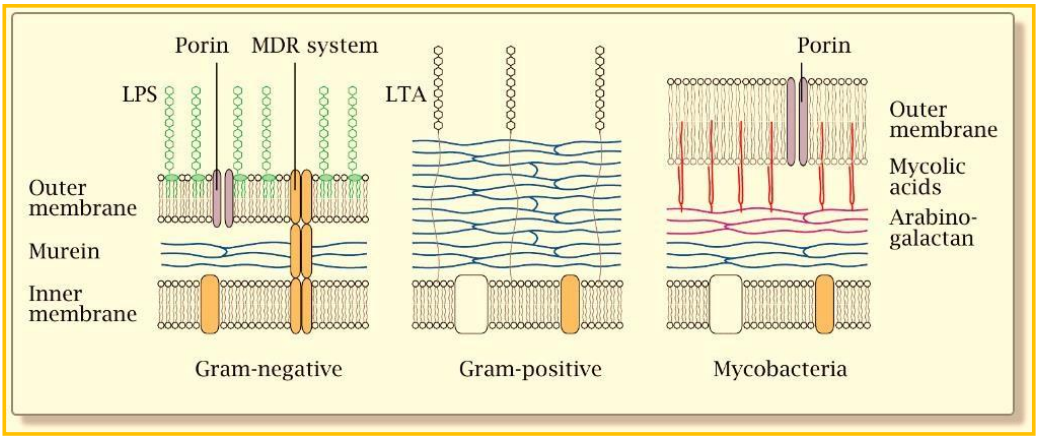
Introduction

The spread of multidrug-resistant bacteria that are causing nosocomial infections is alarming [260]. Many ask the question: "Antibiotic Resistance among Gram-Positive Bacteria in the Hospital Setting: What Can We Do About It?" It seems that the use of agents from the long list of antibiotics [261] is not able to bring remedy to those that got the deadly infection. We examined a possible approach based on antimicrobial peptide [262] surrogates [263] to suggest aid in such a situation [264]. The rational design strategy based on the presumed mechanism of antibacterial effect was adopted to design cationic antimicrobial peptides [265] capable of binding to the bacterial membrane and disrupt it might become advantageous. Then, Proteins and peptides diffusion in the lipid membranes is a crucial aspect of many cellular signaling processes [266]. Those polypeptides reach their goals, these targets could include the outer components of the bacteria, composed mainly of lipopolysaccharide on Gram- negative [267] bacteria and lipoteichoic acid [268] gram (+) bacteria or intracellular components [269], and thereby induce the disruption of the bacteria cells.

The Utility of Antimicrobial Peptides as potential drugs is nowadays recognized by scientists [270]. However, there are a few drawbacks that need the attention of investigators, here are some:

1. Full understanding of how do these AMPs eradicate the microbes. Understanding the selectivity of different AMPs for mammalian and bacterial membranes is of apparent interest in the R&D of these peptides as novel antibacterial agents.
2. Design and synthesis of surrogates to the active moieties present in the naturally present compounds and evaluation of their biological relevance. New classes of antimicrobial agents that do not demonstrate cross-resistance to available agents are desirable for the treatment of these infections.
3. Designing and synthesize selective [271] agents that will allow the eradication of the harming microbes and not harm the microbes that live with us in an essential symbiosis [272].

Previously we have reported on surrogates that are designed on the basis of privileged scaffolds [273].



Bacterial Cell Wall Structure

The AMP surrogate approach has the potential to eradicate the harming bacteria in a non- enzymatic mechanism thereby avoiding many drawbacks that the natural AMP has. For the matter of genetics, the unique mechanism in which bacteria are disrupted, is not influenced by Transduction, Transformation and Cell Conjugation. Therefore, they could be efficient antimicrobial agent also in cases of (MRSA) or Carbapenem-Resistant Enterobacteriaceae (CRE) infections. It seems that today this approach presents one of the very few options to find and develop novel badly needed antimicrobial agents [274].

As primarily described, often the AMP docking [275] molecules are an essential component of the membrane structure. Still, diverse mechanisms of resistance compatible with bacterial survival have been evidenced that directly modify the structure and as a direct consequence. The initiation of the eradication process in all kinds of bacteria starts with the docking of the antimicrobial agent and the outer membrane constructing components. The idea is that binding bacterial eradicating components to a receptor [276] on a

cell surface should mimic the onset of an infection.

The AMP substitutes are designed on the basis of a bi-active motif (Lys-Ala-Ala-Ala-Lys), ie, a wide range of AMPs, amphiphilic and cationic. This has proved to be a broad spectrum antibacterial which does not cause hemolysis of human erythrocytes. We found it in the structure of Dermaseptin S4. Experience with the elimination of Gram-negative bacteria compared to the positive gram teaches us that it is more challenging to treat harmful bacteria caused in comparison with positive bacteria made. This is due to the following reasons:

A) There is a membrane [277] present around the cell wall of the gram (-) which increases the risk of host toxicity but this membrane is lacking positive bacteria caused.
B) Forine channels exist in bacteria causing negative effects which can prevent the entry of harmful chemicals and antibiotics such as penicillin. These channels can also expel the antibiotics and makes it much more challenging to treat compared to the positive bacteria made.
C) Furans are proteins that allow small molecules to pass through the outer membrane; Channel-specific proteins allow other materials to travel through the outer membrane.
D) The risk of resistance [278] against antibiotics is more negative bacteria of Gram due to the presence of external cover around the cell wall.
E) Gram-negative bacteria also have exotoxins and endotoxins but in the case of bacterium positive bacteria have only exotoxins

F) Lipopitheccharide (LPS) component of the outer membrane consists of sugars (O polysaccharides) that act as antigens and fats A (Lipid A is the lipid component of endotoxin responsible for the toxicity of harmful bacteria of the grain), which is endotoxin. Endotoxin causes heat and shock.

G) The outer cell wall protects the cell from phagocytosis and penicillin, lysozyme, and other chemicals Gram (+) cell walls consist of many layers of peptidoglycan and also contain teichoic acids. Teichoic acids may:

1. bind and regulate the motion of cations into and out of the cell
2. prevent extensive wall breakdown and eventual cell lysis during cell growth
3. provide much of the cell wall's antigenicity

Gram-positive bacteria can transport molecules necessary for their survival through holes [279] in their cell wall. The holes in cell walls need to be large enough to let critical nutrients pass through. However, the cell wall must also function to prevent the bacteria's membrane from protruding through a large hole into the environment and lysing the cell [280].

Surface proteins are critical in determining the detection properties of individual bacteria and their relations with the environment. Because the structure of the cell surface is the main characteristic that distinguishes positive gram (+) bacteria, the physical-chemical processes used to transport and attach these proteins show significant differences between these bacterial rates.

The increase in infections caused by pathogenic factors in gram bacteria and the rise of bacterial strains resistant to antibiotics prompted the need for new antibiotics. Recent research indicates that more than 25% of Staphylococcus aureus infections in Europe are caused by MRSA, and most of the isolation is resistant to further antibiotics [281,282].

Gram-negative bacteria are generally less sensitive to inhibitors of cell wall synthesis, which are bacteria with favorable bacteria. The differences between the walls of the cell [of gram positive and negative bacteria] strongly influence the success of bacteria in their environment. A thick cell wall of positive g cells allows them to do better in dry conditions because it reduces water loss. Its external lipopolysaccharide membrane (LPS) helps negative Gram cells excel in the intestines and other host environments [283]. Table 1 summarizes the difference between Gram-negative walls and positive cells.

Table 1: Properties of cell walls. A summary of the differences between gram-positive and gram-negative cell walls [284].

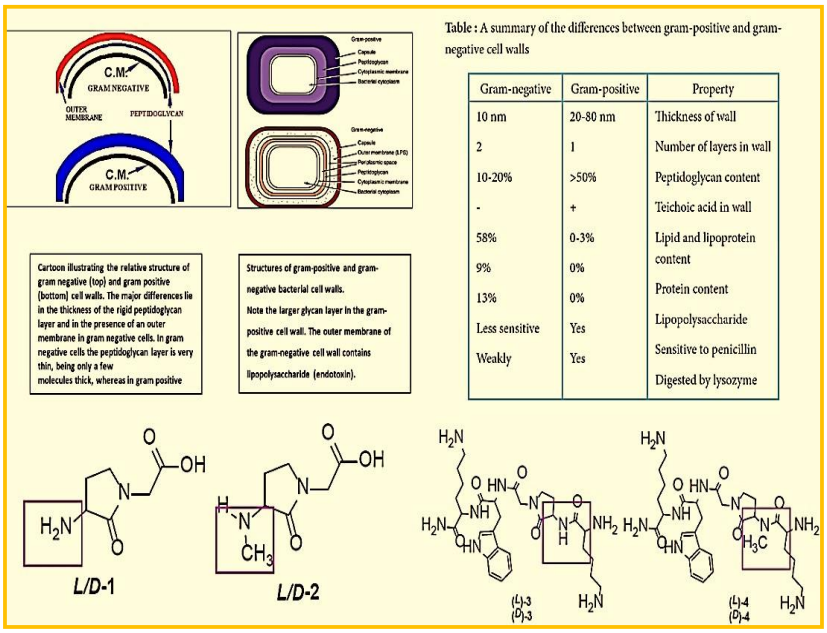
Property	gram-positive	gram-negative
Thickness of wall	20-80 nm	10 nm
Number of layers in wall	1	2
Peptidoglycan content	>50%	10-20%
Teichoic acid in wall	+	-
Lipid and lipoprotein content	0-3%	58%
Protein content	0%	9%
Lipopolysaccharide	0	13%
Sensitive to penicillin	Yes	Less sensitive
Digested by lysozyme	Yes	Weakly

Discussion

The table 2 below reveals an unusual behavior towards the surrogates 30 and 31 compared with 36 and 37. Whereas in the Gram (-) bacteria all surrogates have similar activity for eradication, In the Gram-positive [285] case The N-CH3 analogs (36 and 37), the N-CH3 analogs eradicate easier than in the N-H analogs (30 and 31). This change is made in the Hydrophobic section of the amphipathic molecules of the surrogates (see drawings below, cartoon and table 2) [286]. Modifications in cell wall architecture [287] between the two sorts can enlighten the situation.

Scheme: Bacterial Membranes Cartoon

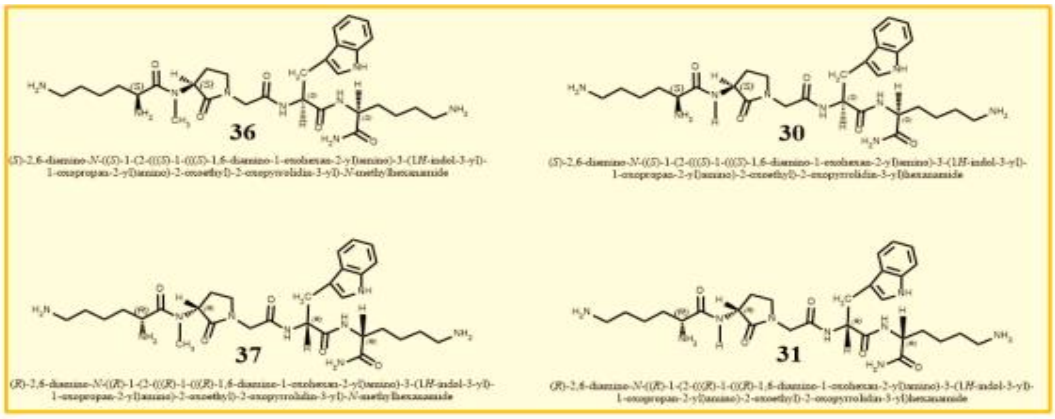
We can compare the attack of a small peptide isolated from honeybees, apidaecin [288]. On bacterial cells as a working hypothesis: a mechanism of action in which the apidaecins kill bacteria bound to the initial ("state surface") non-binding specific of external peptides. Membrane (OM) component. This adhesion is followed by an invasion of periplasmic space, and by a unique and essentially irreversible combination with the receptor/docking molecule it may be an element of permease permeability and transport [289] system on the inner membrane (IM).



Differential eradication compounds

In the final step of penetration, the peptide is translocated into the interior of the bacterium where meets its ultimate target [290].

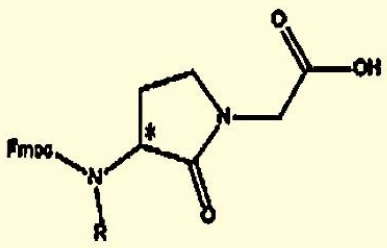
We carried out a comparative experiment designed to examine the effect of N-methylation [291] (N-methylation is a useful technique to modulate the physical-chemicall features of peptides by incorporating one or more methyl groups into the peptidic amide bonds [292]). On the killing of Gram (+) (S. Aureus) and Gram-negative (E. Coli) bacteria and their efficacy. We used the Surrogates in Drawing 1. The outcome is to see in table 2 below:



Drawing 1: Surrogates of *Lys-ala-ala-ala- Lys* used in the experiment.

compound number	COMPOUND SEQUENCE	MIC (μg/ml)		HEMOLYSIS %
		S.Aureu	E.Coli	
30	S-Lys-S-Trp-17-S-Lys	200	100	<3
31	R-Lys-R-Trp-18-R-Lys	200	125	<3
36	S-Lys-S-Trp-19-S-Lys	75	125	<3
37	R-Lys-R-Trp-20-R-Lys	75	135	11

Table 2
The minimal inhibitory concentration (MIC) refers to the lowest coincentratrion of the antibacterial that stops visible growth



One of the primary dilemmas of modern biochemistry is the study of the chemical structure and the functioning of biological membranes, which play a dominating part in the regulation of the molecular and ionic transport between the cell and its environment [293].

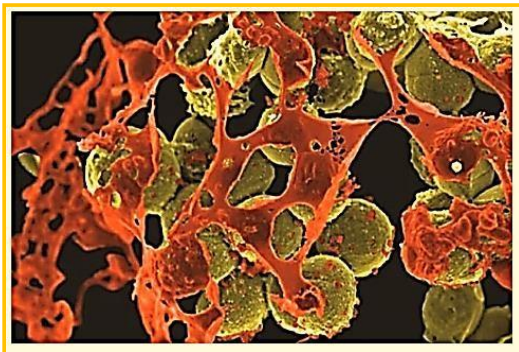
Our testing deals with a case where local structural changes in an agent alter the venerability of one sort of bacteria (Gram-positive) whereas the more robust Gram-negative cell is not affected by this change. This could result from different routes of transport or resistance parameters [294]. Both types of bacteria. (Due to their direct and rapid bacterial activity, it is likely that pathogens acquire resistance against several AMPs derived from short and intense AMP prepared by peptide engineering). Biocides and antibiotics can apparently freely disperse the staphylococcal (positive) wall. The inhibitory and lethal concentrations of many of these antibacterial agents are generally much lower than Gram-negative bacteria, in particular the highly resistant organisms such as P. aeruginosa, Providencia stuartii and Burkholderia cepacia. As such, staphylococcal cells are likely to contain barrier permeability and free uptake of biocides or antibiotics.

Based on research with AMPs, It has been confirmed that docking [295] of AMPs with surface proteins takes place [296]. Mutacin IV (APD Id-AP01174), a 44 aa long peptide derived from Streptococcus mutans UA140, was selected on the grounds of high hydrophobicity to net charge ratio (0.52) and used for in silico docking studies with therapeutically relevant surface proteins. The docking result of IsdB (Staphylococcus aureus IsdB Is a Hemoglobin ReceptorRequired for Heme Iron Utilization.) surface protein and Mutacin IV was found better (ZDOCK score 1168.582) as compared to others [297].

We assume that the bacteria cell envelopes [298] are covered, at the first stage of the eradication, with a carpet [299] of the AMP surrogates. Then the eradication continues. In analogy to polymyxin, defensins, cecropins, magainins, and melittin must cross the outer membrane to reach their final target, the inner cell wall or cytoplasmic membrane (see the cartoon above). As cationic species, they can be expected to bind to LPS; this binding has been demonstrated with defensin [300] and magainin [301].

The state of multidrug-resistant gram-positive organisms is now being overshadowed by the emergence of resistant gram-negative pathogens. In contrast to gram-positive organisms, the pharmaceutical pipeline for antibiotics active against MDR gram-negative organisms is dry with no new medications in advanced stages of clinical development. Due to this emergence of highly resistant pathogens, clinicians have been forced to use antibiotics with known significant toxicities and poorly studied benefits.
Pharmaceutical enterprises have also taken a more active interest in developing antibiotics for methicillin-resistant Staphylococcus aureus (MRSA), rather than gram-negative pathogens [302]. The best reasoning for this imbalance is that MRSA is a leading problem worldwide, whereas the users market for treating gram-negative organisms is smaller, and somewhat more unpredictable given that resistance is rapidly acquired [303].

The most likely explanation for the imbalance in the market. While MRSA has been recognized as a significant problem in hospitals in the developed countries, the market for treating Gram (-) micro-organisms is smaller and somewhat unpredictable given the rapidity of acquisition of resistance [304]. The emergence of Carbapenem-resistant Enterobacteriaceae (CRE) [305]. This has been defined as carbapenem-nonsusceptible and extended-spectrum cephalosporin-resistant Escherichia coli, Enterobacter aerogenes, Enterobacter cloacae complex, Klebsiella pneumonia, or Klebsiella-oxytoca. Some exclude ertapenem resistance from the definition, which is based on Gram-negative microbes. This composition focuses attention on the eradication of Gram-negative bacteria [306].



A new CDC report is bringing a lot of attention to the emergence of drug-resistant bacteria in hospitals and long-term care centers across the country. Carbapenem-resistant Enterobacteriaceae, or CRE, strike critically-ill patients in nursing homes and intensive care units where infections can be fatal in up to 50 percent of cases, according to the report.

CRE bacteria: The next superbug threat in your hospital.

The resistance to carbapenem is not the only reason CRE bacteria are considered dangerous. CRE bacteria that reach the bloodstream have a mortality (death) rate of 40%-50%. CRE is transmitted, usually by direct contact with contaminated feces, skin [307], or instruments used in hospitals.

Most resistant bacteria are gram-positive. The cell wall of the gram (+) and the outer membrane of Gram-negative bacteria contain lipid and anion molecules. In gram-positive bacteria, it is lipo-teichoic acid (LTA-teichoic acid) and lipopolysaccharide (LPS) that may compete with the plasma membrane for interaction with AMP (see caricature). Not only the cell walls, but then also the inner plasma membrane are targets for its AMP or Surrogate. The matrix is generated by a different phospholipid bilayer in the head group and the vehicle composition acid contributes to a mechanical variety of AMPs against microbial cells. We have noticed that the elimination of Gram-positive bacteria (S. Aureus) affected the introduction of the N-CH3 transformation to the molecule and increased the killing ability by a factor of three. As in AMPs, Chirality is not expected to make any difference in the elimination of bacteria.

Rational

The eradication of the Gram Negative bacteria is essentially the same in all 4 surrogates (Table 2 above). The alternations in the characteristics of the molecular introduced by N- methylation [308] do not affect the way the surrogate kill the Gram-negative bacteria. In contrast, the eradication of the gram-positive is enhanced by this change (from MIC 200 to MIC 75)

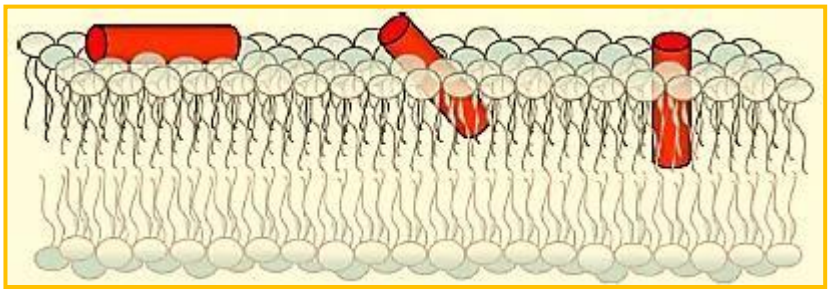
There are necessarily two pathways that antibiotics can take through the outer membrane [309]

1. a lipid-mediated pathway for hydrophobic antibiotics, and
2. general diffusion porins [310] for hydrophilic antibiotics.

One of the determining events is entering the bacterial membrane [311].

The membrane disassembling by antimicrobial agent involves at least three steps: A membrane disorder by an antimicrobial agent involves at least three stages [312]:

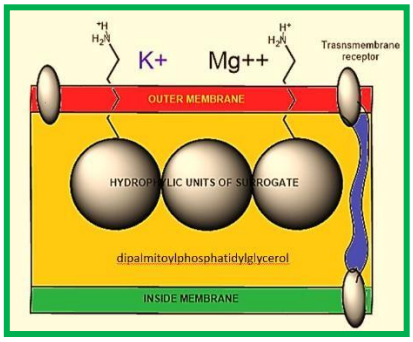
1. First, a cationic peptide can detect coat and the surface of anionic bacteria. With the classical amphipathic helical structure, this cation peptide prefers to target the anionic bacterial membrane.
2. Second, the agent binds to the external membranes and crosses the outer membrane.
3. Third, the peptide reaches the inner membrane. This initially binds to the parallel membrane surface membrane, which is the basis for the carpet model at high concentrations, and the peptide may disrupt the membranes by micellization. Alternatively, the peptide may take a vertical position to form a pore.

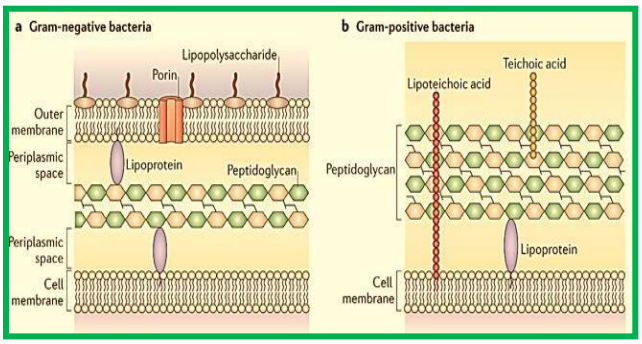


Association of amphipathic α-helical peptides (cylinders) with a lipid bilayer can occur in three general orientations: parallel to the membrane surface, at an oblique angle, or perpendicular to the membrane surface (i.e., along the bilayer normal).

The transport of solutes through to the inner or cytoplasmic membrane of bacteria usually takes place through specific active transport systems (for instance "wormholes [313]") that require energy [314]. The transport of the peptide-mimics from the on the outer membrane organized carpet to its destination in the inner cell membrane is different regarding the two sorts of bacteria. We estimate that the penetration of the molecule into the membrane of the bacteria is different in the two types: Whereas penetration [315] of the peptide mimic to the Gram-negative bacteria needs a high energetic [316] effort due to the crowded situation used by stacking it with membrane proteins and lipoproteins [317],

demanding a high energy track for both types of the surrogates where the slight change in energy needed is too small compared to the overall penetration energy. At any case, there is an energy need for such a membrane deformation needed in the penetration of the surrogates into the inner membrane.





Surrogate enters the inner membrane and snorkels out the lysine amino groups to replace metal ions and disintegrate the membrane.

Recently, evidence was provided that eradication takes place only when bacterial cell membranes are wholly saturated with AMPs. This condition is achieved for all bacteria. However, Since the in Gram (-) bacteria the outer membrane are crowded, packed with various proteins (up to 50% of the whole membrane weight [319]), compared of only 15% in the S-layer (surface layer) [320] in gram (+), it demands more energy for saturation in gram- negative than in gram-positive (see table 1 above).

The bringing in of the N-CH3 unit to the Penta peptides surrogates stiffens the structure thereby causes an increase in energy demand for saturation. The fraction of this energy in Gram-negative is smaller than in Gram (+) due to membrane packing composition. It is easier for the N-CH3 to penetrate the outer membrane in gram-positive bacteria and in Gram- negative. Since saturation is achieved in gram-positive with fewer energy demands, the Gram-positive compared to gram-negative are eradicated in preference (by "snorkeling" [321]322 for example).

The Conclusion from The Experiment Of N-Methylation

The Gram-positive bacteria are more comfortable to penetrate [323] since the outer membrane is weak in membrane proteins. In such an event, small energy changes can become significant for the travel of the agent into the outer membrane. The N-CH3 are less flexible and therefore penetrates more accessible to the membrane. Finally, after the surrogates are located in the inner part of the outer membrane, the Lys □-amine unit can "snorkel" [324] out and disassemble the membranes of both Gram-positive and Gram- negative bacteria. The interactions of an AMP with the cell wall cannot be explained by a particular sequential amino-acid pattern or any motif; instead, they originate from a combination of physicochemical and structural parameters [325] including size, residue composition, overall charge, secondary structure, hydrophobicity and amphiphilic nature [326,327].

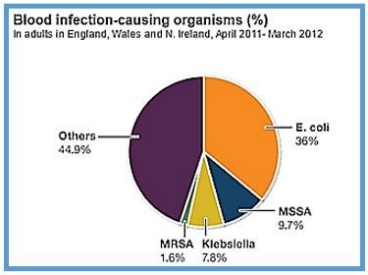
The "drilling of pores" by interaction of AMP's with the cell wall lipids have been considered to rationalize the difference in activity of Nisin [328] and Magainin [329] (Magainin is at least two orders of magnitude less active), it was assumed that the changes in permeability [330] and with it the ease of penetration of the AMPs through the formation of the pores is determining the effect on the eradication difference which is a result of disintegration of the plasma membrane of the bacteria [331].

The interactions of an AMPs surrogates with the membrane cannot be explained only by a particular sequential amino-acid pattern or motif; sooner, they originate from a combination of physicochemical and structural features [332] including size, residue structure, overall charge, secondary structure, hydrophobicity and amphiphilic character [333]. Also interactions with the many components that furnish the architecture of the membranes are crucial. From our experiment we conclude that the venerability of bacteria may depend on small structural variation in the composition of the biocide.

Among the gram-positive organisms, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus-faecium* (VRE) represent the most significant therapeutic challenges.

The danger posed by growing resistance to antibiotics should be ranked along with terrorism on a list of threats to the nation, the government's chief medical officer for England has said.

http://www.bbc.com/news/health-21737844



New Drugs Needed

Human medicine has reaped the benefits of antibiotics for the past 80 years, but without urgent action, the utility of these agents will be drastically minimized. To limit the spread of resistance, physicians must use antibiotics judiciously and apply infection-control procedures consistently. These measures alone, however, will not be sufficient. New antibiotics are desperately needed. To address this emerging crisis, the medical community, governments, pharmaceutical companies, and public health agencies must all work together. Only a coordinated and committed response can slow the rising tide of multidrug-resistant bacteria.

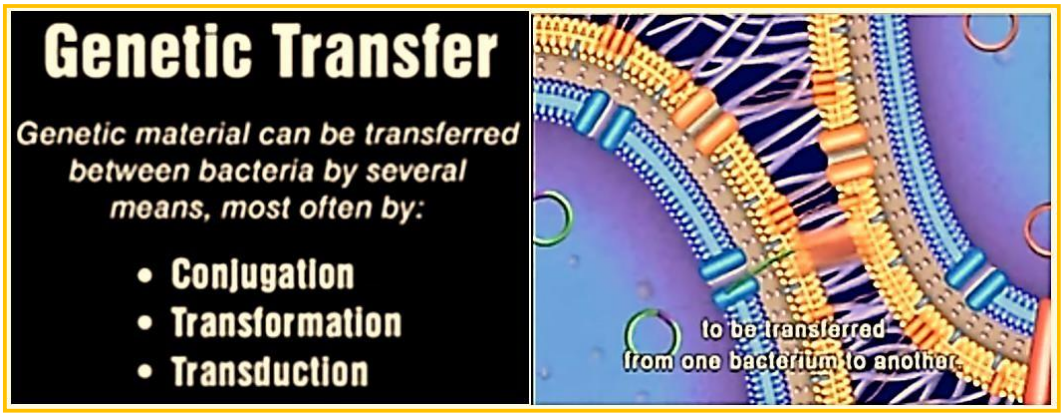
Morbidity of CRE

From the descriptive point of view, among patients who received combination treatment, mortality was up to 50% for the tigecycline-gentamicin combination, up to 64% for tigecycline-colistin, and up to 67% for carbapenem-colistin. Among the monotherapy-treated patients, mortality was up to 57% for colistin and up to 80% for tigecycline. Specific regimens were administered to a small number of patients in individual studies

Antibiotics Were So Last Century! War On bugs Like MRSA To Be Fought With Tetraspanin Proteins?

Scientists have warned doctors and patients for many years that the end of the era of antibiotics was over. Bacteria have become resistant to even recent antibiotics and resorts in some regions of the country and around the world. Something as simple as an infected scrape or a before can kill. While Americans were in a state of panic over measles, which reportedly kills less than one American a year, a deadly monster known as MRSA lives under their noses. MRSA is a durable drug called the full story shared from Apple News.

• https://www.youtube.com/watch?v=Zxi8xyeZXHk



"A particularly important type of surface is that of a biological membrane, and understanding the interaction of proteins and peptides with biological membranes is important for the development of novel antimicrobials, the treatment and prevention of diseases, and the preservation of biological membranes under highly stressed conditions"

http://www.physics.uoguelph.ca/dutcherlab/ProteinPeptide.shtml

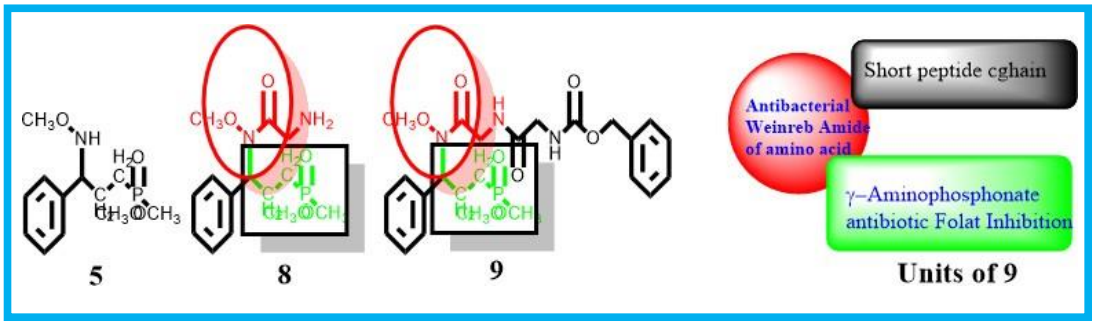
Preparation of γ-(N•methoxy)-amino-phosphonic Acids dimethyl esters as Precursors to Biomimetic Peptides [334]

Abstract

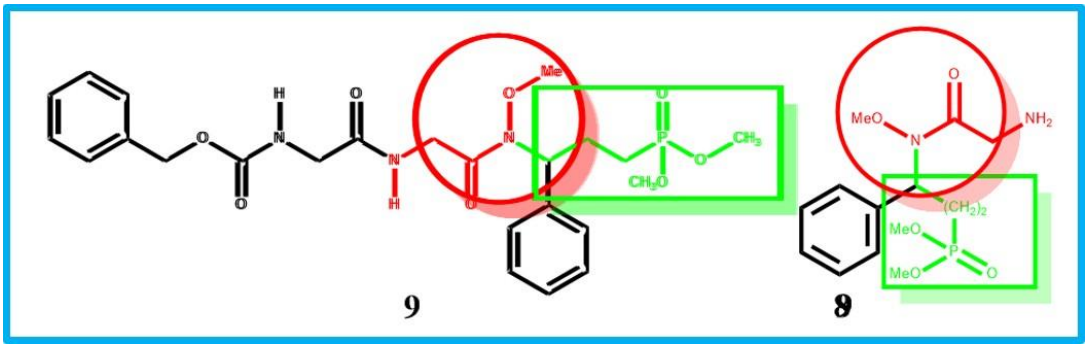
Phosphono-1-N-methoxyamine acids may function in potential as useful biomimetic derivatives of natural amino acids and as a source for biomimetic peptides. A synthetic approach is presented herein for the preparation of y-phosphono N-methoxy amino acids 5 and a protected dipeptide namely ***benzyl (2-((2-(methoxy(3-(methoxy(oxo-l6-methyl)phosphoryl)-1-phenylpropyl)amino)-2-oxoethyl)amino)-2- oxoethyl)carbamate 9.*** γ-amino-phosphonates

may be applied in folate (antibacterial, anticancer) research. The research effort on the subject of synthesis and biological value of g-amino phosphonates is being pursued in many places. The structure of our target molecule 9 has a Weinreb type amino acid amide moiety and a γ-amino-phosphonate unit as a structural building block. Although Weinreb amides (see Drawing 1 and 2 below, red section) and γ- amino-phosphonates (green section) may operate in different molecular mechanisms, the synergy between the two moieties may introduce a remarkable antimicrobial effect in 8 and 9.

Keywords: Synthesis, Biomimetic, Peptides, Precursors, Amino phosphonates.



Drawing 1: Schematic design; Introducing N-Methoxy-γ-amino phosphonates into tripeptide mimics γ-amino-phosphonates (folic acid bio-synthesis inhibitor) and Weinreb amides of amino acids are antibacterial components.



Drawing 2: The target molecules of this research.

Introduction

The fatal nosocomial pandemic is the cause of hospital mortality mainly through deadly infections caused by new strands of bacteria that are resistant to contemporary antibiotic drugs. Peptides and their mimics have recently become one of the main topics of interest in chemistry and biochemistry, aiming at elucidating bioactive peptides and understanding their function and mode of action. Synthetic analogs, containing phosphorous and boron derivatives or organometallic units, for example, of the natural amino acids and peptide moieties are needed in the process of evaluating the structure-activity relationship (SAR) of peptides and of the corresponding peptidomimetic analogs [335-355].

Polypeptides of amphibian origin like South American tree frogs (Cationic peptide isolated from skins of American tropical frog Phyllomedusa Sauvage [356,357,358,359]) exhibit diverse biological activity and short fragments are a promising potential for novel very deserved antimicrobial drugs [360-366]. Approximately 40,000 harmful and/or lethal hospital errors occur each and every day in the US. The Hygiene at the healthcare facilities should be enhanced with more efficient antimicrobial agents, phosphonates [367-372] might be suitable materials.

However, a famous water pollutant is phosphate [4d], water-softening mineral additives that were once widely used in laundry detergents and other cleaners. When phosphates enter waterways, they act as a fertilizer, spawning overgrowth of algae. This overabundance of aquatic plant life eventually depletes the water's oxygen supply, killing off fish and other organisms. Although many states have banned phosphates from laundry detergents and some other cleaners, they are still used in automatic dishwasher detergents. Phosphonates are similar to phosphates except that they have a carbon-phosphorous (C-P) bonding place of the carbon-oxygen-phosphorous (C-O-P) linkage. Due to their structural similarity to phosphate esters, phosphonates often act as inhibitors of enzymes due in part to the high stability of the C-P bond. In nature, bacteria play a major role in phosphonate biodegradation. The first phosphonate to be identified to occur naturally was 2-aminomethyl phosphonic acid [4e,f].

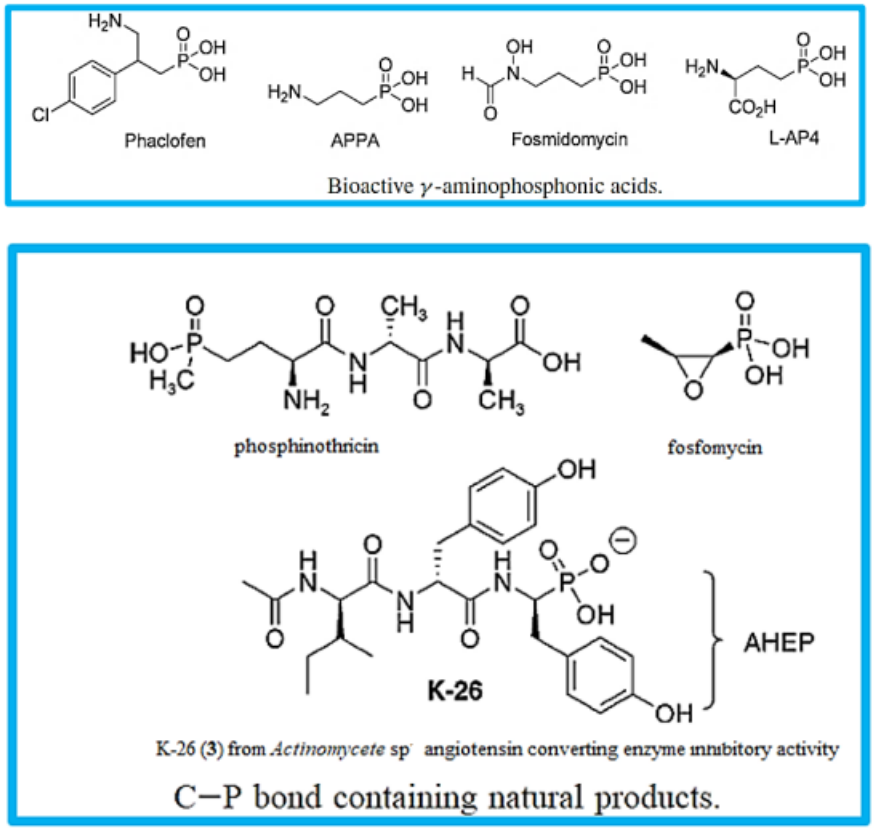
One of the promising approaches to combat this nosocomial pandemic is the utilizing of phosphonic acid moieties, present in many agricultural applicable agents. We have shown before that ultrashort fragments of Dermaseptin S4 are very potent antibacterial substances [1p]. The mono isopropyl-amine salt of Glyphosate is present as the active ingredient in the widely used

herbicide Roundup®. Glyphosate and its natural product analog phosphinothricin inhibit the shikimate pathway of aromatic amino acid biosynthesis via the enzyme 5-enol-pyruvyl shikimate-3-phosphate (EPSP) synthase (3-phospho-shikimate-l- carboxyl vinyl-trans)- phrase [373,374,375,376,377]. It was reported that Interaction of the herbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3- -phosphate synthase in atomic detail.

Although the phosphonic and carboxylic acid groups differ considerably with respect to shape, size, and acidity, amino phosphonic acids are considered to be structural analogs of the corresponding amino acids and the transition state [1l,378,379,380,8c,17] that mimics reversible peptide hydrolysis. In this communication, we have pursued our effort of finding novel antibacterial agents in short peptide surrogates [1,d,o,p]. For this we utilized oxime ethers, for the preparation of short peptide based on N- methoxy amide [381,382,383,384,385] combined with phosphonic acid moieties.

Some on the Biological activity of Synthetic amino phosphonates

Some phosphorous peptides display significant neurophysiological effects. Dipeptides containing phosphonic acid analogs of glycine and β- alanine are strongly antagonistic to NMDA, inhibiting NMDA-evoked responses in the pentapeptides, phosphonic analogs of enkephalins, exhibit analgesic activity comparable with, or stronger than, that of their opiate counterpart [386,387,388], to novel β-lactamase inhibitors (BLIs) bearing an electrophilic center (phosphonates, aldehydes, trifluoromethyl ketones, and boronic acids) that can covalently modify the nucleophilic catalytic serine is conceptually advancing our understanding in this field [389,390]. A large variety of chemical modifications of peptides is commonly used in this regard, such as elimination and addition of one or more amino acid residues, isosteres [391,392,393,394,395,396] to the peptide bond [1a,b], etc.

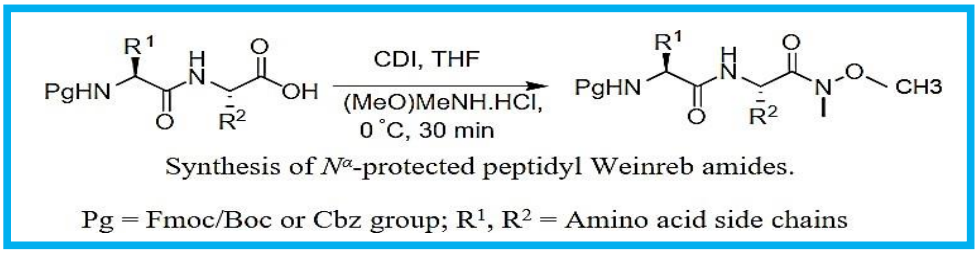


Drawing 3: Natural phosphorous based bioactive compounds (credit ref. [10d]).

One significant modification that constrains peptides is the N-methylation of the nitrogen atom of the peptide amide. Many such N- methyl substituted natural peptides have been isolated from microorganisms and vegetables. Peptide-surrogates contain "unnatural" amino acids as building blocks. N-methyl peptides show antibiotic and antituhowevermor activities and immunosuppressive effects. [397,398,399,400]. Such peptides were reported by Gilon and co-workers as analogs of Cholecystokinin and as N-methyl SP3 analog. N-methylation causes a markable conformational change in the peptide mimics. It was shown that N-methylation might promote the eradication of some bacteria. [401-404]. Recent work from the Leibniz Institute of Plant Biochemistry [12d], shows that a set of N-alkylated peptide derivatives were screened against Aliivibrio fischeri, but only the (N-methylated) natural product displayed noteworthy activity of ca. 40 μM IC50, independent of stereochemistry. The electron-donating property of the - CH3 group might be considered. In such circumstance, the -OCH3 unit might increase such electron donation to the amide bond [405-411,34]. N-Methoxy-N-methyl amide, popularly addressed as the Weinreb amide, has surfaced as an amide with a difference, they

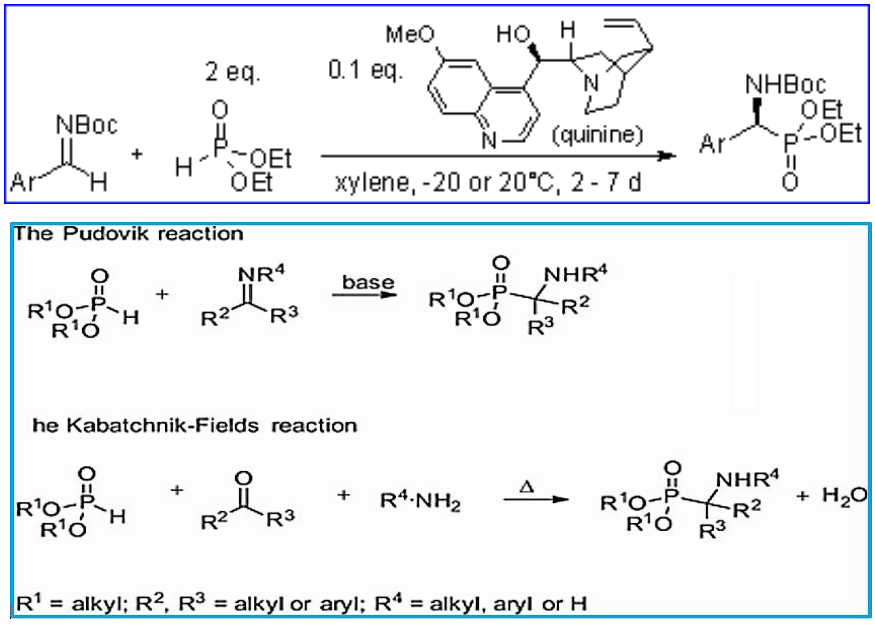
exhibit antimicrobial bio-activity [13b]. The Weinreb amides were subjected to in silico studies, to predict the preferred orientation and binding affinity between the molecules using scoring functions. s. Based on the minimum binding energies, antibacterial activities have been conducted for a number of the synthesized compounds. The antibacterial results of Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. Based on the docking results the N-Fmoc-L-Ala-N(OCH3)CH3 and N-Fmoc-L-Phe-N(OCH3)CH3 were showing good activity in in vitro studies .

Herein, we report an efficient, one pot synthesis of Nα-protected amino acid/peptide acid-derived Weinreb amides employing N,N'-carbonyl diimidazole (CDI [10a]) as the activating agent. The prepared compounds were screened for in silico molecular docking studies and in vitro antibacterial activities [13c]. Antibacterial activity was screened by the Agar well diffusion method [13c,] against three pathogenic bacterial strains, Escherichia coli, Pseudomonas aeroginosa and Staphylococcus aureus (one Gram +ve and two Gram –ve). This amide has served as an excellent acylating agent. Pakistani and Indian scientists [13b-d] report on the antibacterial activity of alanine and phenylalanine derived Weinreb amides against different bacterial strains [13b,c].

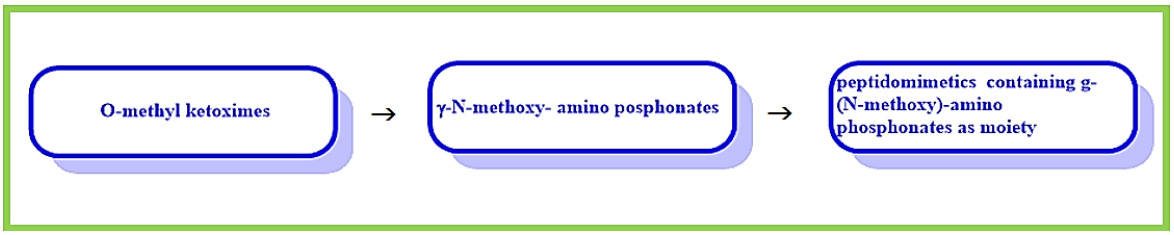


Drawing 4: Antibacterial Weinreb amides of some amino acids (credit ref. [13b]).

Also, modification of peptides consists of changing the carboxylic group to its roster- a phosphonic acids [412-416] unit may enhance activity (the □-N-substituted amino phosphonate can be prepared in a modified Kabachnik-Fields Reaction [417]). These compounds are structural analogs of amino acids in which a carboxylic moiety is replaced by phosphonic acid or related groups [418,419]. Acting as antagonists of amino acids, they inhibit enzymes involved in amino acid metabolism and thus affect the physiological activity of the cell. These effects may be extended as antibacterial agents, plant growth regulatory materials or neuromodulators. They can act as ligands, and heavy metal complexes with amino phosphonates have had medical applications investigated.

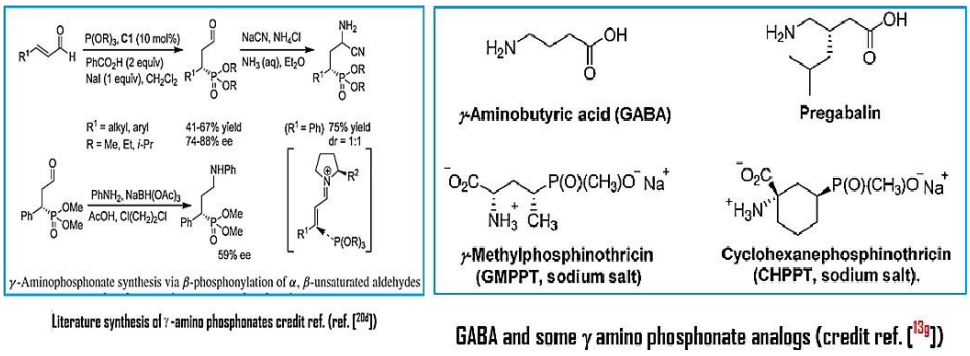


Drawing 5: Amino phosphonate synthesis by the Kabachnik-Fields and Pudovik Reactions (credit ref. [15a, 420]).



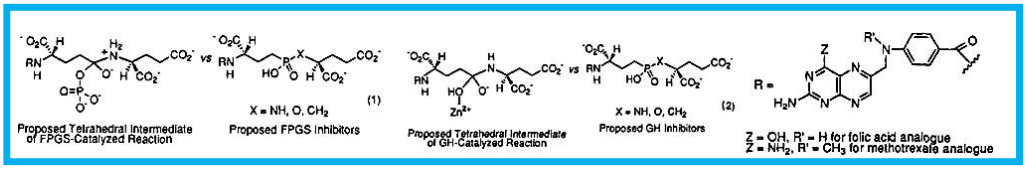
Drawing 6: Scheme 1: General strategy for the synthesis.

Amino Phosphonic acids were used as bioactive materials [421-424], as well as analogs representing transition states of the group. The biosynthesis of poly- □-glutamyl peptide [16b] derivatives of folic acid and related anti-folate drugs such as the applied drug methotrexate (MTX) involves a non-ribosomal ATP-dependent reaction catalyzed by folylpolyglutamate synthetase (FPGS). Research has demonstrated that this reaction proceeds via a γ-glutamyl phosphate of reduced folate or MTX which then reacts with an incoming molecule of L-glutamate to form a new glutamyl- glutamate peptide bond [13c].



Literature synthesis of γ-amino phosphonates credit ref. (ref. [20d])

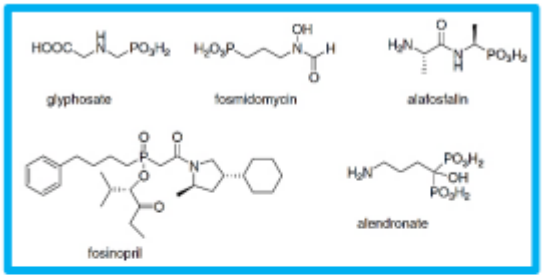
GABA and some γ amino phosphonate analogs (credit ref. [13g])

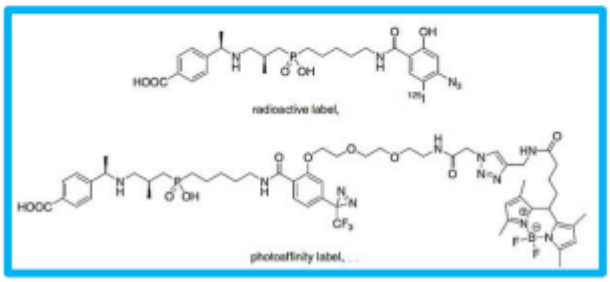


Drawing 7: y-amino-phosphonates in research (credit ref. [17c]).

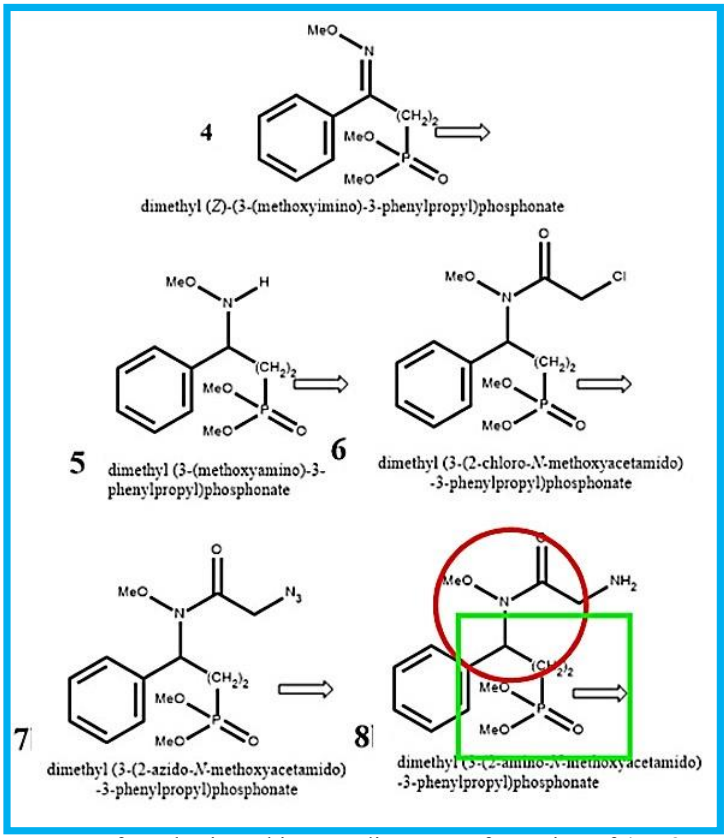
Amino phosphonic acids (present in K -26, in Baclophen phosphonate analogs such as Phaclofen, CGP 54626, CGP 35348, and the alendronate, a bisphosphonate medication used to treat osteoporosis and Paget disease, bone diseases [10d]) and synthetic modifications show neurologic, antibacterial, antibiotic and antitumor activities as well as the herbicides and fungicides activities [425,426]. Differential Inhibition by amino phosphonates was reported [427-429]. Gamma-amino phosphonates are reported to serve as the bio-isosteric analogs of gamma- aminobutyric acid (GABA) [19d]. Gamma (γ)-Aminobutyric acid (GABA) has been shown to be an important central nervous system (CNS) neurotransmitter. The properties of amino phosphonates as transition state analogs of amino acids, and as anti-bacterial, antifungal and anti- HIV agents, attracted considerable attention. γ-Amino phosphonic acid in particular is a bioisosteric analog of GABA (γ-aminobutyric acid).

Acting as antagonists of amino acids, they inhibit enzymes involved in amino acid metabolism and thus affect the physiological activity of the cell. These effects may be extended as antibacterial, plant growth regulatory or neuromodulators, as well as analogs representing transition states of enzyme-substrate interactions. This was done with the purpose of understanding the mode of action of competitive inhibitors in biological systems [9a,b]. It was the purpose of the present research to synthesize γ-(N-methoxy) amino-γ-substituted phosphonic acids and to show the feasibility of using these acids as precursors for phosphonic acid-containing biomimetic peptides.



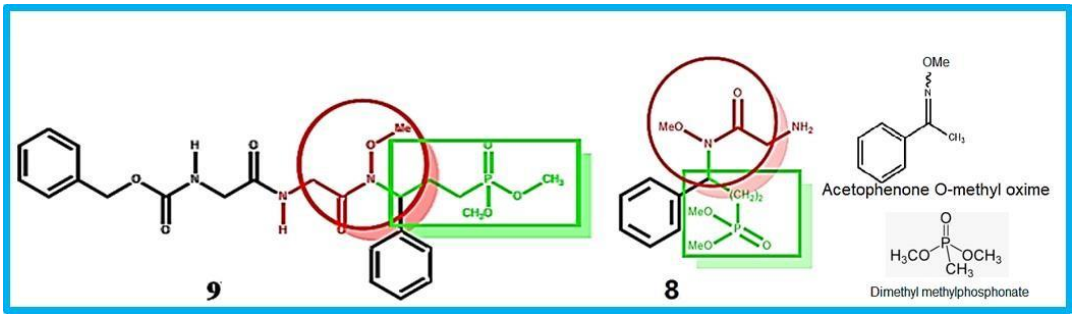


Results



Drawing 8: Targets of synthesis and intermediates transformation of 4 to 8.

Coupling of 8 with N-Cbz-glycine affected by DCC resulted in the derived biomimetic peptide ***9***.

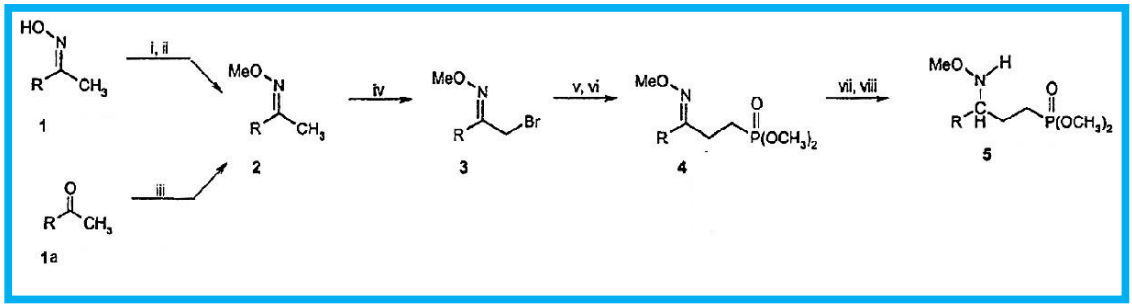


Drawing 9: Acetophenone O-methyl oxime and Dimethyl methyl phosphonate applied for the preparation of the bioactive Weinreb amides phosphonates 8 and 9.

The synthesis of the target class of compounds, outlined in Drawing 9 is based on the chemistry of oxime ethers which was intensely studied in our laboratory.[430] The starting materials for the synthesis were oximes l and the ketones la which were converted to the corresponding oxime ethers 2 by either direct oximation using methyl hydroxylamine hydrochloride or by a two-step oximation reaction [431-433]. Subsequent α-bromination of these oxime ethersusing N-Bromo succinimide [434-436].

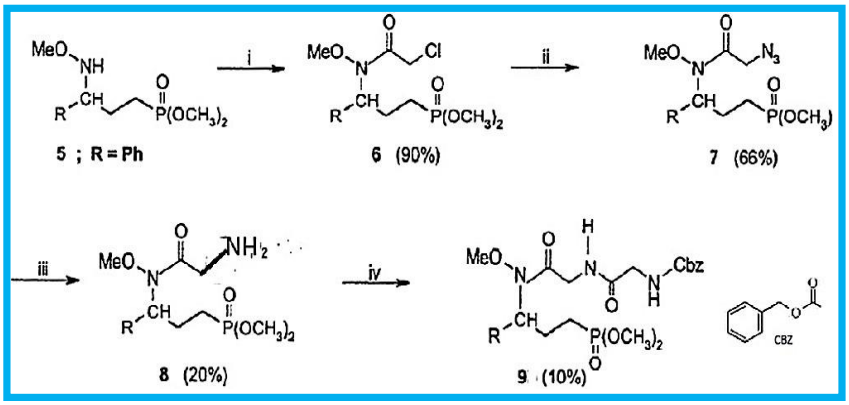
Synthesis of 9

The starting materials for the synthesis were oxime l and the ketones la which were converted to the corresponding oxime ether 2 by either direct oximation using methyl hydroxylamine hydrochloride or by a two-step oximation reaction[437].to yield the target class of compounds, namely the *dimethyl (3-(methoxamine)-3- arylpropyl) phosphonates 5.*



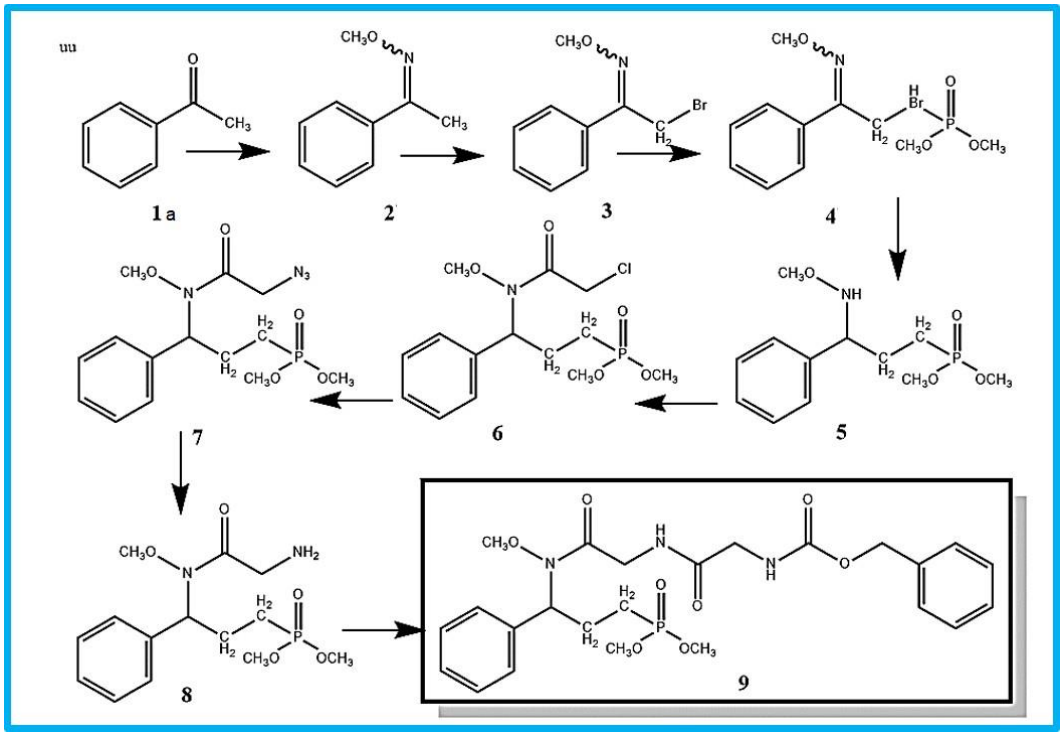
Drawing 10: Synthesis of dimethyl (3-(methoxyamino)-3-phenylpropyl)-phosphonate-arylpropyl)- phosphonates.

The feasibility of using this new class of □ -(methoxy) amino phosphonic acids 5 as potential precursors for biomimetic peptides is demonstrated by the preparation of a derived biomimetic dipeptide-dimethyl.3- Phenyl-3(N-methoxy-Naminoacylation)-1-propyl phosphonate 5 (Drawing 10). Attempts to affect the coupling of the substrate 6 with N- Cbz- glycine using the DCC-HOBT or BOP-Cl coupling agents were unsuccessful. This difficulty was bypassed by chloro-acetylation [438] of 6 to yield the chloro-acetyl derivative 6 (recently this strategy was also applied to the preparation of as syn-bimane containing tripeptide surrogate agent that can cross the Blood Brain Barrier into the animal's brain from the bloodstream [439-441]. This was done by the use of the chloro-acetyl chloride and substitution of the chlorine to the azide 7 (5→6 →7 [442]). Reduction of the azide group of 7 with Pd-CaC03/H2 [443] afforded the target amino derivative 8 [27,444].



Drawing 11: Synthesis of benzyl (2-((2-((3-(dimethoxy-phosphoryl)-1-phenylpropyl) (methoxy)amino)-2-oxoethyl)amino)-2-oxoethyl) carbamate 9.

Scheme legend: Reagents and conditions for the conversion of 5 to 8 and 9:6b, i 10% NaOH-H 0, 19% NaCO3 -H 0, CICH2CH2Cl, r.t., 30 min, extraction (CH2Cl2); ii , NaN3-DMF, 0°C, Sb-DMF, r.t.,3hr; iii , 7 CH30H, Pd/CaC03 (cat.), H2, 24 hr; iv, 8, HOBt, N-Cbz-glycine, THF, DCC, 0°C, 60 min, r.t., overnight.



Drawing 12: Synthesis of 9 by the oxime ethers route.

Conclusion

As our research program demanded, we continued our work towards examining a simple synthetic procedure to achieve a tripeptide surrogate for the testing of the biological feasibility for the eradication of bacteria. We have thus continued with the intermediate 4 aiming at 9 for the eradication test.

The C=N double bond of the O-methyl-oxime group was reduced with various hydride agents, the best was sodium cyanoborohydride in acetic acid to yield 5.

The reaction with chlorine acetyl-choline [445] afforded 6. Reaction with NaN3 in cis-2,6-dimethyl-piperidine (cis-DMP) gave the azide 7 and hydrogenation gave the amino compound 8. Subsequently, the peptide bond formation afforded 9.

Phosphonates are a class of materials that are utilized in the agriculture-intensive farming methods as herbicides, fungicides as for example.

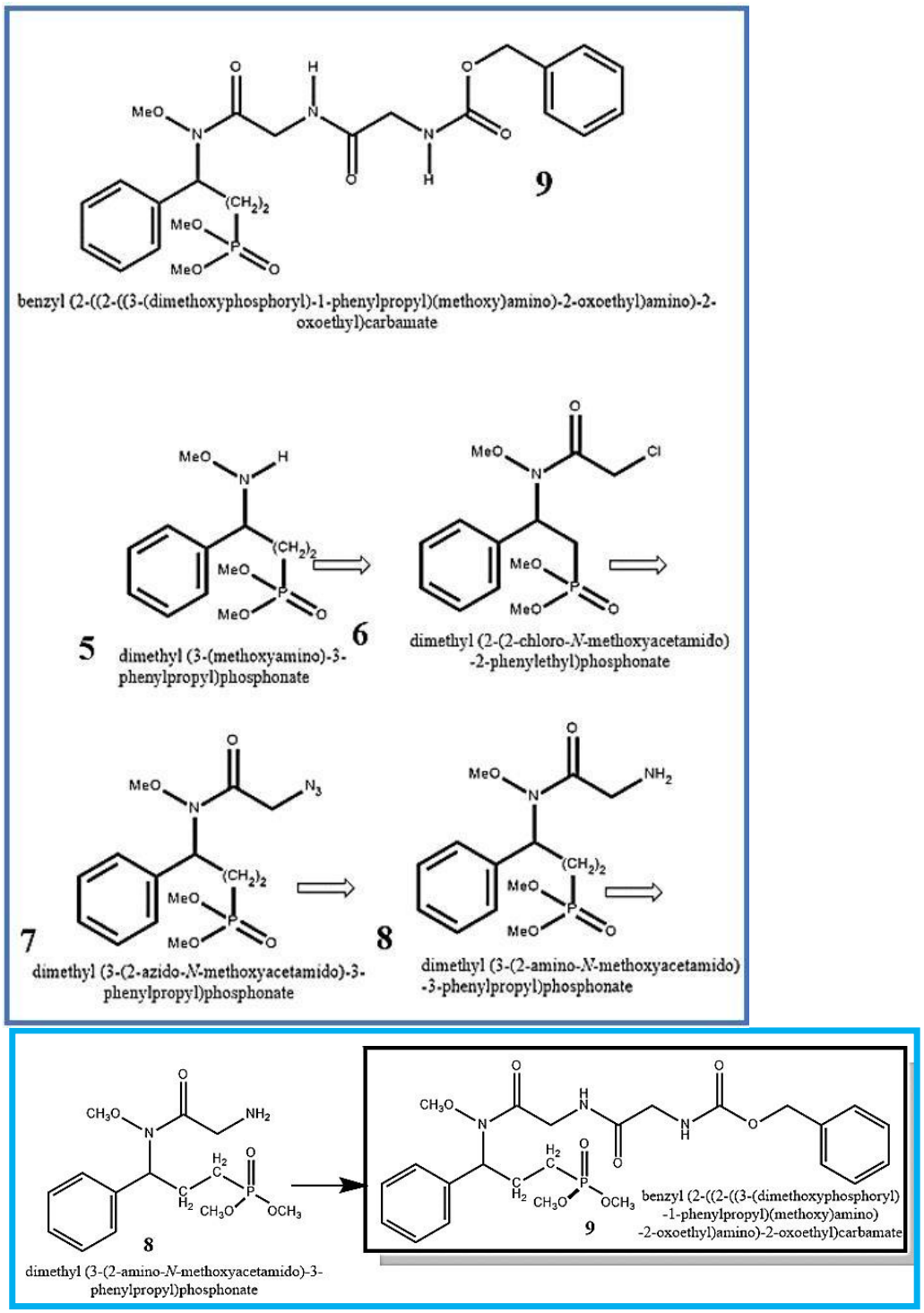
Biological Activity

In our project we were looking for a microbial agent. In early testing, we tested our compounds for E. coli G and Staph (Aureus G+) vaccines of phosphonates 5, 6, and 7, but almost the biological activity was observed in the elimination experiments only at a high molten concentration.In these compounds, the only part that is known as an antimicrobial entity is the phosphonate unit.

Although the agents 8 as well as 9 are cis-2,6-dimethylpiperidide (cis-DMP) [446] results that do not indicate selectivity.

Our preliminary tests show that 8 and 9 exhibits very similar antibacterial activity, suggesting that the combination of the two pharmacophores may be necessary to eliminate the bacteria. This may indicate that the increased glycine supplementation in CBZ may be unnecessary in relation to antimicrobial activity. In addition, some amino phosphonates [447,448], for instance benzothiazole phosphonate derivatives, also possess the ability to cross the blood-brain barrier in vivo mice studies and thus hold great potential for inner brain therapy. It is published that antibiotic-induced perturbations in gut microbial diversity influence neuro-inflammation and amyloidosis in a murine model of Alzheimer's disease [449,450,451,452]. The struggle with the into the brain infiltrating Gut Microbes might be a new focus for Alzheimer's therapy.

However, the N-OMe (Weinreb amide) and the □-amino-phosphonate units are needed for the eradication of the bacteria. Although a weak antibacterial activity was detected, we concluded our project with this result.



1H-NMR, 13C-NMR, 31P-NMR, IR. MS spectra and elemental analysis.

Table 1: Physical Data for the sequence of compounds 4→9.

compound#	IR (KBr)	^{1}H-NMR	^{13}C-NMR, δ^{31}P	Analysis	MASS
4	1050, 1170, 1250, 1620 and 2950 cm·';	δH: 2.02 (2H, m), 2.97 (2H, m), 3.72-3.78 (6H, d,J=10.8), 3.99 (3H, s), 7.37 (3H, m), 7.62 (2H, m);	δc: 19.3, 19.9-22.8, 52.15, 61.9, 125.9, 128.4, 129.1, 134.5, 156.2; δp: 33.49;	Calcd for: $C_{12}H_{18}NO_4P$ C, 53.14; H, 6.69; N, 5.16; O, 23.59; P, 11.42 Found C 53.45; H,6.87; N, 5,50, P, 11.24	m/z: 271
5	3600,2203,1603, 1475,1405,1380, 1170,1065,910	δH: L57 {2H, m), 3.47 (3H, s), 3.7 (6H, d, J=10.8), 3.69 (2H, m), 3.9 {lH, m), 7.28 (5H, m);	δc: 21.9, 26.47, 52.2, 62.56, 65.7, 127.6, 127.9, 128.6; dp: 34.17	Calcd for: $C_{12}H_{20}NO_4P$ C, 52.74; H, 7.38; N, 5.13; O, 23.42; P, 11.33 Found C, 52.55; H7.54; N 5.45, P11.07	m/z: 274
6	1050, 1200, 1700 and 3000 cm·	δH 1.79 (2H, m), 2.41 (2H, m), 3.49 {3H, s), 3.76 (6H, d, J=8.I), 4.08 (2H, s), 5.46 (1 H, m), 7.327.43 (5H, m);	δc: 22.34, 30.36, 41.63, 53.44, 61.59, 65.89, 127.0, 128.9, 129.4, 169.0; δp: 34.615	Calcd for: $C_{14}H_{21}ClNO_5P$ C, 48.08; H, 6.05; Cl, 10.14; N, 4.00; O, 22.87; P, 8.86 Found C, 48.23; H, 5.98; Cl, 10,45, N, 3.76; P8.65	m/z: 318;
7	3250-2203,1050, 1700, 2200, 3000 and 3400	δH: 1.49 (2H, m), 2.52 (2H, m), 3.46 (3H, s), 3.77 (6H, d, J=8.2), 5.46 (1 H, m), 6.46 (2H, s), 7.44-7.66 (5H, rn);	δc: 22.2, 30.5, 53.2, 61.6, 65.7, 128.1, 129.1, 129.4, 130.1, 139.5, 168; δp: 34.56;	Calcd for: $C_{14}H_{21}N_4O_5P$ C, 50.91; H, 7.02; N, 8.48; O, 24.22; P, 9.38 Found: C, 51.o3; H, 7.34; N, 8.40; P9/17	m/z 356
8	3250-2203 1050, 1750 and 3200-4000 cm·	H 1.48 (2H, m), 2.52 (2H, m), 3.43 (3H, s), 2.78 (6H, d, J=8.1), 5.43 (lH, m), 6.45 (2H, s), 7.43-7.65 (5H, m);	δc: 22.2, 30.45, 53.1, 61.6, 65.7, 128.1, 129.1, 129.3, 130.0, 139.4, 168; /δp: 34.6.	Calcd for:$C_{14}H_{23}N_2O_5P$ C, 50.91; H, 7.02; N, 8.48; O, 24.22; P, 9.38 Found: C, 51.09,; H,6.78; N, 8.32; P, 9.45	m/z=330
9	3250-2203,1600, 1575,1400,1210, 1160,1055,915	δH. 1.08-1.90 (4H, m), 3.32-3.47 (2H, m), 4.06-4.16 (12H, m), 5.13 (2H, d), 5.44-5.67 (4H, m), 7.18-7.84 (lOH, m);	δc: 23.9, 41.3, 48.24, 54.9, 64.05, ?C 110.01, 116.45, 124.6, 125.3, 126.57, 126.9, 127.3, 128.9, 131.1, 173.95; δx: 34.6	Calcd for $C_{24}H_{32}N_3O_8P$: C, 55.28; H, 6.19; N, 8.06; O, 24.54; P, 5.94 Found:C, 55.03, H, 6.42, N, 8.33, P, 6.23	m/z=521

Cyclic Peptides based on Analogs of Dermaseptin S4 Fragments [453]

Abstract

A cationic peptide isolated from skins of American tropical frog Phyllomedusa Savage may offer a basis for design and synthesis of antimicrobial therapeutics. The presence of the positively charged amino acid lysine in the sequence of the natural product supplies the moieties essential for the biocide activity by the disintegration of the outer cell wall. Cyclic analogs [454] based on the basic structural features of the natural cationic peptides did not improve the biocide properties as compared with their linear analogs.

In this work, we tried to explore a ring effect on the activity of small antimicrobial peptides. The synthesis and hemolysis, as well as antibacterial activity as broad-spectrum bactericides, is reported.

Introduction and Background

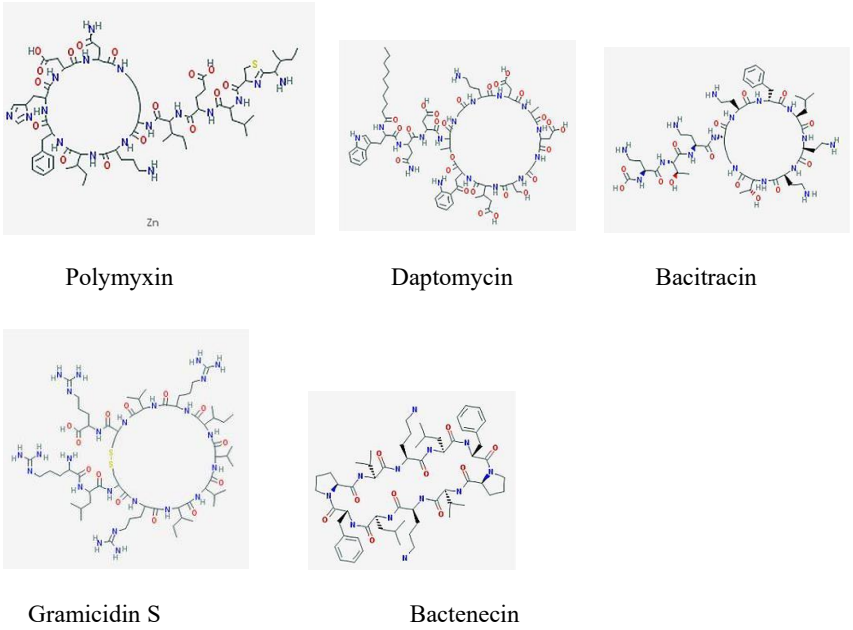
It has been reported that small cyclic antimicrobial peptides disrupt membrane function by integrating into the lipid bilayer [455]. Clinical studies recommend cyclic antimicrobial peptide rhesus θ-defensin-1 (RTD-1) is a promising potential therapeutic agent for cystic fibrosis airway disease. RTD-1 is a recently discovered cyclic peptide that, like other well-studied antimicrobial peptides, appears to bind to the lipid matrix of the cell membrane in the initial stage of activity. It is far from certain that the molecular mechanism of õ-defensins will follow the same pattern as magainin and protegrin. The richness and versatility of peptide-membrane interactions prompted us to study this novel cyclic peptide RTD-1[456].

The ability of microbes to develop resistance towards antimicrobial agent demands the search, design, and preparation of medicinal treatment to combat the severe life-threatening problem. Recently, The World Health Organization published a report focusing on antimicrobial resistance and global surveillance with the request to develop novel drugs and treatment for the healthcare of the world's population [457]. In the last century, the antibiotics revolution contributed by eradicating many bacterial infectious diseases (Cholera, Syphilis, Pneumonia, and Tuberculosis for example), to the increase of human life longevity in a dramatic manner. It also contributed to wartime to treat the wounded of WWII from deadly infections. However, the built-up of microorganism resistance was always a determining cause to look for better and more effective agents for the eradication of microbes.

Natural products, in particular, those that protect the living organisms from the invasion of microbes and parasites were one of the central guides for the drug developing people, as is the immune systems found in all kingdoms of living creatures. In these innate immune systems are materials that contain the means for the antimicrobial combat and defense 458.

In particular tropical frogs from the Phyllomedusa [459] family contain in their antimicrobial skin peptides, Mor [460] and collaborators thoroughly investigated Dernaseptin S4 (NH2-Ala-Leu-Trp-Met-Thr-Leu-Leu-Lys-Lys-Val-Leu-Lys-Ala-Ala-Ala- Lys-Ala-Ala-Leu-Asn-Ala-Val-Leu-Val-Gly-Ala-Asn-Ala-COOH). (ALWKTLLKKVLKAAAKAALKAVLVGANA), as a representative of this biologically active set of cationic peptides. On the other hand, Juan R. Granja [461] concluded in his publication that "Cyclic peptides may be useful since they may disintegrate the living microbial cell." The ability of cyclin D, L-α -peptides, and the related β -amino-acid cyclic peptides to form a wide array of structurally analogous is remarkable. These are biologically active structures, with surface properties that are tailored individually and coupled with the ease of the peptide synthesis. They are expected to open new opportunities in the treatment of existing and emerging infectious diseases through both rational design and selection from combinatorial cyclic peptide libraries. Cyclic cationic adopt unique conformation and interaction of the antimicrobial peptides in lipid bilayer [462].

Today, some of the most effective antimicrobial drugs are based on cyclic peptides; Examples are Polymyxin, Daptomycin and Bacitracin [463], Gramicidin S [464] and Bactenecin [465].



Polymyxin Daptomycin Bacitracin

Gramicidin S Bactenecin

Cyclic Antimicrobial cationic peptides 466.

Rationale and Aim

Wu and Hancock and collaborators [8,467] observed in their work on open-chain and cyclic Bacitracin that behavior of the cyclic form (S-S bond exists to form the macrocyclic ring system) and the open chain form (S-S bond is reduced) that there is a remarkable difference in antimicrobial effectiveness. The medium leads the researchers to differentiate between two mechanisms of action: The outer cell membrane is corrupted by the positively charged ends of the cationic amino acids in both cases. However, the cyclic form can form channels in the cell membrane [468] and thereby enter to the inner cytoplasm. Their internal cell processes may take place such as corruption of the genetic materials of the microorganism. The cyclic form is, therefore, more potent than the open chain in Bacitracin.

The discovery of a new family that consists of Short Cyclic Antimicrobial Peptides, the Ranacyclins [469] gives hope for selective eradication of bacteria lines. A possible mechanism for their biological activity was suggested in which a possible mechanism of pore formation by ranacyclins and pLR. Peptides bind in the first step predominantly by hydrophobic interactions and align parallel (stacking) to the outer membrane surface. Increasing peptide concentration results in the insertion of the peptides into the hydrophobic core of the membrane to form transmembrane pores. The authors suggested that these cyclic antimicrobial peptides may bear the fundamentals of selectivity in targeting the bacteria lines to be eradicated at a later step of the biological activity.

Ranacyclins

Bearing this in mind, we were interested in exploring the possibility to obtain from the linear peptides Dermaseptin S4 and its shorter derivative cyclic peptides and to learn in preliminary experiments about the ability to the linear cationic peptides in the cyclic version to eradicate both Gram + as well as Gram – Bacteria. We also were interested to know if the cyclic compounds will affect human red blood cell hemolysis.

The purpose of the study and its importance

The general research objective is to develop cyclic peptides based on Dermaseptin S4 with
Inherent antibacterial properties

✓ Synthesis of peptides aged 9-11 amino acids, linear and cyclic

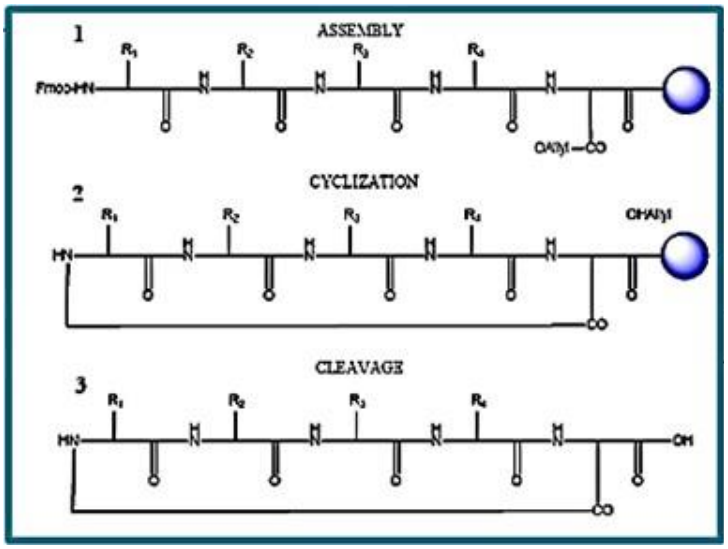
✓ Checking activity on bacterial Gram + and Gram – and reaction towards human red blood cells hemolysis.
Results

Although it is accepted in the literature that It is microbicide for bacteria and fungi at low micro molar concentrations. Antibacterial activity of the cyclic peptide was threefold more considerable than that of an open-chain analog [470a]. Cyclization can also constrain a bioactive peptide in its active conformation, thus lowering the entropic cost of binding to its biological target [14b]. Similarly, Stelsed [471] and collaborators studied the mechanistic significance of the cyclization on the biological activity in the eradication of both grams negative and gram- positive bacteria. Thus, it was investigated by comparing the antibacterial activities of the cyclic peptide to that of thetri-disulfide-containing acyclic analog from which it was produced. The peptides were tested for activity against S. aureus and E. coli in an agar diffusion antimicrobial assay. It was found that the cyclized peptide was three times as active as the acyclic analog against both organisms, indicating that cyclization confers a substantial increase in antimicrobial potency. Mitsuzaki [472] and colleagues reported that the cyclic -sheet antimicrobial peptide tachyplesin I (T-SS) was found to show a 280-fold higher affinity for lipopolysaccharides (LPS) compared with acidic phospholipids, whereas the linear helical peptide F5W-magainin 2 (MG2) could not discriminate between LPS and acidic phospholipids.

The controversy about the properties of cyclic vs open chained bactericide peptide led us to inquire about the activity issue.
We examined the antibacterial and report on the synthesis and efficiency of some 30 cyclic peptides and learn about the structural, including chirality, on their antimicrobial utility.

The general strategy for the preparation of linear and cyclic peptides on reliable support (SPPS) (carboxylic and amine in side chains form the cyclic structure by the production of a new amide bond)

The strategy is based on a stepwise buildup of the linear peptide chain on reliable support (Rink Amide), removal of the F-moc protection of the NH2 terminus, removal of the allyl ester and then cyclization on the reliable support followed by cleavage of the cyclic-peptide from the reliable support



Scheme 1: Strategy for the synthesis of linear and cyclic cationic peptides

a) Chemical Synthesis: Cyclic Peptide synthesis [473].

Linear Peptide synthesis: Linear Peptides were prepared in a reactor on a solid substrate (Fmoc Rink-Amide MRHB resist). Ay first, the preparation of the linear peptides was carried out. Then, the bulk was divided into two batches. The resis was downloaded in separate reactors one for the linear and the other for cyclization [474].

We have used F-moc, t-Butyl and allyl [475] as protection for residue protection.

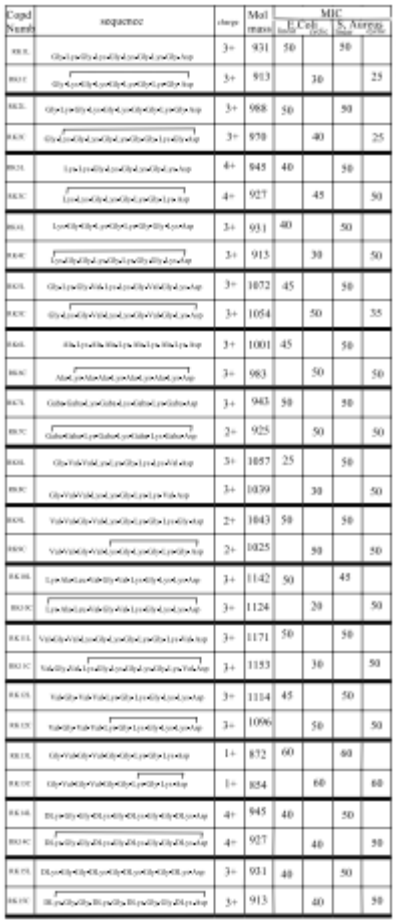
For the Lys side chain amino group, we applied the carbonyl tert-butyloxy. (Boc) amino acid -Fmoc-Asp-OAL first Resin added to allow cyclization before removal from the solid phase. Removal of the F-moc protection group was carried out by a solution of 20 % piperidine in DMF 2x10) for 10 minutes. Washing was done by using N-Methyl Pyrrolidone (NMP) for 3 times each 5 minutes and Dichloromethane (DCM) for 2 times each of duration of 5 minutes in an alternating manner. Upon completion of the connection the t-Bu ester protecting group was removed by treatment with Pd (PPh3) 4 0.1mmol in DCM-AcOH-MMP (37:2:1) under an atmosphere of nitrogen for two hours on the resin bearing the peptide.

The linear peptide was washed with DCM and NMP - then washed twice with 20 % piperidine in DMF and another rinse with NMP and DCM. NMP-resin slurry was separated into two reactors. Linear peptides were obtained by final Fmoc removal.

Application of the Head to side chain Cyclization strategy for the synthesis of cyclic peptides from the linear peptides [7, 476]: The cyclization was carried out by treatment with PyBOP, 1mmol HOBt 1mmol and DIEA 2 mmol with 6 ml of NMP in the reactor. The changed reactor was connected to a shaker for one hour. We then rinsed with NMP) three washes for five minutes each (and DCM 2) drops to five minutes each (Off. Remove Resin peptides made using trifluoroacetic acid (TFA)-H2O-Triisopropylsi lane (TIS) (95:2.5:2.5) for one hour Cocktail Shaker and excess liquids were removal by nitrogen stream followed by washing with diethyl ether and decantation gave the following products, they were kept frozen
at -4ºC until further use. The following were prepared:

b) The following compounds were prepared and their biological activity as bactericides was examined using the MIC method [477] as follows (Table 1).

Table 1: Linear peptides and their cyclic analogs MIC comparison E.Coli and S.Aureus.



c) Inspecting the data presented in table 1 leads us to the following conclusion:

✓ The existence of Lysine (or Arginine) units in all peptides are essential to the bactericidal properties of all peptides. One can locate such sequences in the **Ranacyclins T** and **E**.

✓ The ratio between cationic amino acids to hydrophobic may vary from 10:90 to 90:10 cationic vs hydrophobic amino acids in the peptide. No remarkable effect was detected on the grounds of MIC results between cyclic and their linear analogs. However, the eradication of both Gram+ and Gram- bacteria is observed by all substances tested without much difference linear or cyclic structure.

✓ Comparing the bactericidal effect of the cyclic peptides with that of short linear fragments (5 amino acids short peptides) of Dermaseptin S4 reveal that there is no remarkable change in the biological activity. It seems the presence of the Lysine side chain is essential for the bactericidal activity of all the compounds synthesized and tested [478].

It seems that the mechanism in which antimicrobial peptides eradicate bacteria depends mainly on secondary peptide sequences rather that spatial architecture as chirality or cyclic constructs. The positively charges amino acids (Lys, Arg) including the location of those in the framework of the bioactive peptide or surrogate determine the mode and efficiency of the eradication process.

R.S. Hodges [6,479] and collaborators investigated the role interactions of the cell membrane with cyclic antimicrobial cationic peptides and presented graphic description on the of cyclic Structure and amphipathic topology of the cyclic cationic antimicrobial peptides. The L-Lys and D-Lys residues are denoted by K and K, respectively. From inspecting this presentation, the -CH2- NH2 groups present in our structures of the cyclic and linear antimicrobial peptides, is interacting with the outer cell membrane. This may be the cause in our investigation for the bactericide activity, The lysine NH2 groups are disintegrating the cell membranes in a similar manner in linear and cyclic peptides investigated in this paper.

Combatting Antimicrobial Resistance [480]

• Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi.

• AMR is an increasingly severe threat to global public health that requires action across all government sectors and society.

• Without effective antibiotics, the success of major surgery and cancer chemotherapy would be compromised.

• The cost of health care for patients with resistant infections is higher than care for patients with non-resistant infections due

to longer duration of illness, additional tests and use of more expensive drugs.

• In 2016, 490 000 people developed multi-drug resistant TB globally, and drug resistance is starting to complicate the fight against HIV and malaria, as well.

Antimicrobial resistance happens when microorganisms (such as bacteria, fungi, viruses, and parasites) change when they are exposed to antimicrobial drugs (such as antibiotics, antifungals, antivirals, antimalarials, and anthelmintics). Microorganisms that develop antimicrobial resistance are sometimes referred to as "superbugs".

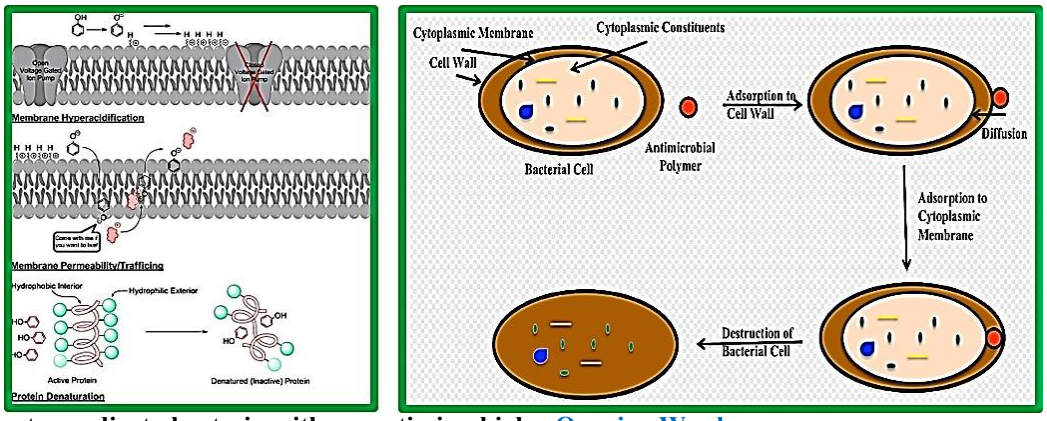
As a result, the medicines become ineffective and infections persist in the body, increasing the risk of spread to others.

Opening Words

The human health problem that annoys the population of earth these days due to the resistance and character-change of many bacteria strands due to horizontal gene transfer is threatening public health,. The nosocomial infections situation is alarming, one of 25 entering a healthcare facility gets infected and one of nine dies from the infection. Antibiotic resistance is a growing public health problem. The United Nations recently acknowledged this as "one of the biggest threats to modern medicine," dedicating a high-level meeting to the issue at the 2016 General Assembly [481]. The infectors are now everywhere in the public domain, but mainly in lavatories, restaurants, recreation parks for example, everywhere, and this overflow of infectors is the next even more alarming stage of the pandemic. There is an urgent need to harness forces for the combat with the microbes, with the hope to win this battle. Hand wash may help, but more thorough hygiene is needed, even in the soil. The current regulatory environment is a further factor constricting research and development of antibacterial agents by large and small pharmaceutical companies. Regulatory authorities are less prepared to accept adverse side-effects with antibiotics than other classes of therapeutic agents and demonstration of superiority is required for new antimicrobials. Both these factors are likely to contribute to a further decline in pharmaceutical industry involvement in the discovery and development of new antibacterial and antiseptic agents.

Challenge

One major world problem, the microbe's pandemic, should be addressed in the biblical way. Erase the old and look for new, state of the art antimicrobial [482] and antiseptic agent.



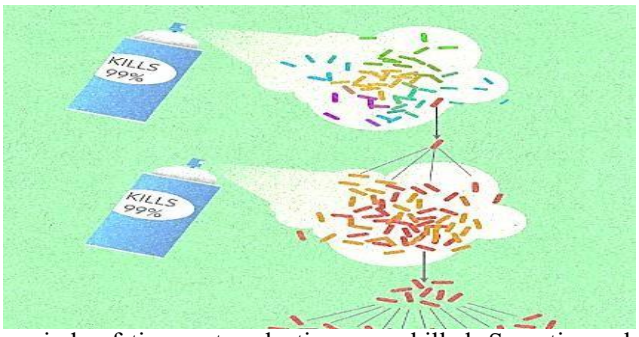
General pathway to eradicate bacteria with an antimicrobial agent.

We humans have adapted to live in harmony with different microorganisms throughout evolution, this balanced symbiotic relationship can sometimes shift and allow pathogenic bacteria to blossom and cause infections. In the struggle for survival, a complex mechanism involving many key components assists in the elimination of these infectious agents.

The problem's focuses are many [483], here we mention three target areas: Health (Hospital Acquired Infections, HAI), Food sterilization and Agriculture [484] (Livestock, soil sterilization).

Opening Words

Antimicrobial agents have changed human and animal health systematism by revolutionizing our weapons in the war against infectious disease, resulting in improved survivability. There was a time where the perfect antimicrobial drug "The magic bullet a search for the perfect drug" was considered a n achievable target [485] for both human and animals. However, this health triumph has been tempered by the subsequent realization that bacterial populations can quickly modify them to resist [486].



Frequent antibiotic use over long periods of time puts selective pressure on bacteria, and causes resistance to spread. When an antibiotic is used to treat a typical bacterial infection, most bacteria are killed. Sometimes, however, a bacterium with an advantage lives. This bacterium can then reproduce and pass its advantage on, creating many more antibiotic resistant bacteria.

Antibiotics in the clinical pipeline in 2015
MS Butler *et al*

Table 1 Antibiotics and β-lactamase inhibitor combinations launched from 2000 to 2015, their antibiotic class, activity spectra, country of first approval, lead source and NP lead source if applicable

Year approved	*Drug name*[a,b]	*Class*	*Bacteria type*	*Country of first approval*	*Lead source*	*NP lead source*
2000	Linezolid	Oxazolidinone	G+ve	USA	S	
2001	Telithromycin	Macrolide	G+ve/G−ve	Germany	NP-derived	Actinomycete
2002	Biapenem	Carbapenem	G+ve/G−ve	Japan	NP-derived	Actinomycete
2002	Ertapenem	Carbapenem	G+ve/G−ve	USA	NP-derived	Actinomycete
2002	Prulifloxacin	Fluoroquinolone	G+ve/G−ve	Japan	S	
2002	Pazufloxacin	Fluoroquinolone	G+ve/G−ve	Japan	S	
2002	Balofloxacin	Fluoroquinolone	G+ve/G−ve	South Korea	S	
2003	Daptomycin[b]	Lipopeptide	G+ve	USA	NP	Actinomycete
2004	Gemifloxacin	Fluoroquinolone	G+ve/G−ve	USA	S	
2005	Doripenem	Carbapenem	G+ve/G−ve	Japan	NP-derived	Actinomycete
2005	Tigecycline	Tetracycline	G+ve/G−ve	USA	NP-derived	Actinomycete
2007	Retapamulin[b,c,d]	Pleuromutilin	G+ve	USA	NP-derived	Fungus
2007	Garenoxacin	Quinolone	G+ve/G−ve	Japan	S	
2008	Ceftobiprole medocaril	Cephalosporin	G+ve/G−ve	Canada	NP-derived	Fungus
2008	Sitafloxacin	Fluoroquinolone	G+ve/G−ve	Japan	S	
2009	Tebipenem pivoxil	Carbapenem	G+ve/G−ve	Japan	NP-derived	Actinomycete
2009	Telavancin	Glycopeptide	G+ve	USA	NP-derived	Actinomycete
2009	Antofloxacin	Fluoroquinolone	G+ve/G−ve	China	S	
2009	Besifloxacin[d]	Fluoroquinolone	G+ve/G−ve	USA	S	
2010	Ceftaroline fosamil	Cephalosporin	G+ve/G−ve	USA	NP-derived	Fungus
2011	Fidaxomicin[b]	Tiacumicin	G+ve	USA	NP	Actinomycete
2012	Bedaquiline[b]	Diarylquinoline	G+ve (TB)	USA	S	
2012	Perchlozone (1)	Thiosemicarbazone	G+ve (TB)	Russia	S	
2014	Delamanid (2)	Nitroimidazole	G+ve (TB)	Europe	S	
2014	Dalbavancin (3)	Glycopeptide	G+ve	USA	NP-derived	Actinomycete
2014	Oritavancin (4)	Glycopeptide	G+ve	USA	NP-derived	Actinomycete
2014	Tedizolid phosphate (5)	Oxazolidinone	G+ve/G−ve	USA	S	
2014	Ceftolozane (6)+tazobactam[e] (7)	β-Lactam+β-lactamase inhibitor	G−ve	USA	NP-derived/NP-derived	Fungus/actinomycete
2014	Nemonoxacin (8)	Quinolone	G+ve/G−ve	Taiwan	S	
2014	Finafloxacin[d] (9)	Fluoroquinolone	G+ve/G−ve	USA	S	
2015	Ceftazidime[e] (10)+avibactam[b] (11)	β-Lactam+DBO β-lactamase inhibitor	G−ve	USA	NP-derived/S	Fungus
2015	Ozenoxacin (12)[d]	Quinolone	G+ve	Japan	S	

Abbreviations: G−ve, Gram negative; G+ve, Gram positive; NP, natural product; S, synthetic; TB, tuberculosis, USA, United States of America.
[a]The structures of the antibiotics approved from 2000 to 2012 can be found in our previous reviews.[2,3]
[b]First member of a new antibiotic or β-lactamase inhibitor class approved for human therapeutic use.
[c]Pleuromutilin derivatives have been previously used in animal health.
[d]Approved for topical use.
[e]Tazobactam and ceftazidime were first launched in 1992 and 1983, respectively.

The short list in the table above represents some of the newest antibiotic drugs approved for antiinfection treatment. Please notice the poor yields in antibacterial agents in the last decades, only a score of novel drugs was found and approved [487], do not provide weapons to fight infections in healthcare installations (nosocomial infections) and give only little hope to the millions suffering from the fact6 that they do not provide remedy to those that are infected with the resistant microbes and in most cases, must die while treated in hospitals.

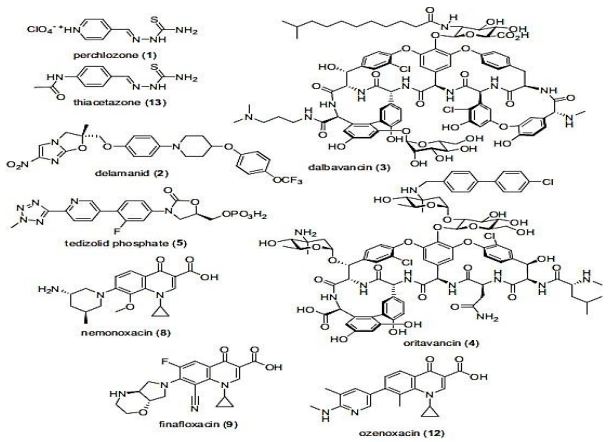
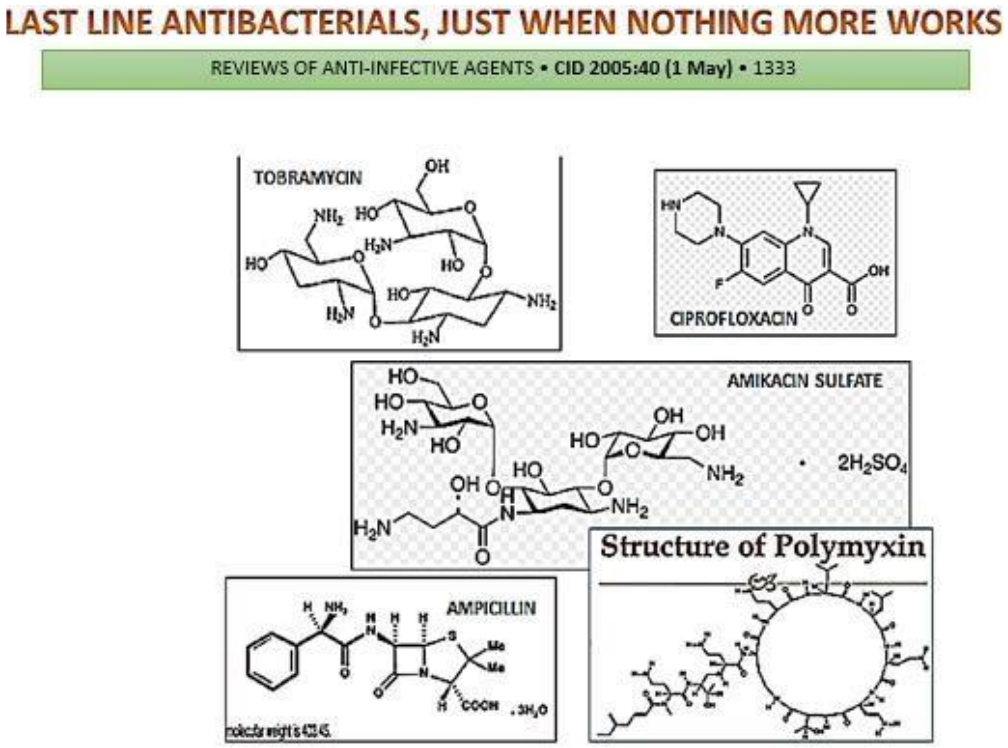


Figure 2 Structures of the recently launched antibiotics, perchlozone (1), the 1950s TB drug thiacetazone (13), delamanid (2), dalbavancin (3), oritavancin (4), tedizolid phosphate (5), nemonoxacin (8), finafloxacin (9) and ozenoxacin (12).

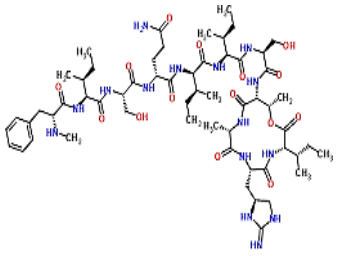
Figure 2: Structure of recently launched antibiotics, perchlozone (1), the 1950s TB drug thiacetazone (13), delamanid (2), dalbavancin (3), oritavancin (4), tedizolid phosphate (5), nemonoxacin (8), finafloxacin (9), ozenoxacin (10).

One of the reasons is that even the most modern findings in the area are impotent in the killing of bacteria when resistant strands are the matter.

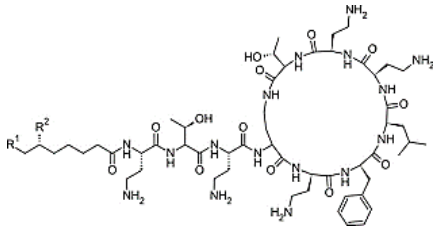


Colistin (polymyxin B) was re-introduced as "last resort" for treating patients with acute infection based on drug resistant bacteria, However established that the cyclic peptide is very harmful to central organs like heart, liver, kidney brain and is a lethal substance in a bout 60% of its use causes mortality 488Polymyxins, a group of polypeptide antibiotics that consists of 5 chemically different in 1947. Only polymyxin B and polymyxin E (Colistin) have been used extensively worldwide in Colistin was discovered in 1949 and was non- ribosomally synthesized by Ba5]. Coli of colistimethate sodium in 1959. However, the m due to multidrug-resistant (MDR), gram-negative bacteria in patients with cystic fibrosis.

However, the emergence of bacteria resistant to most classes of commercially available an- tibiotics and the shortage of new antimicrobial agents with activity against gram-negative microorganisms have led to the reconsideration of polymyxins as a valuable therapeutic option.



Teixobactin



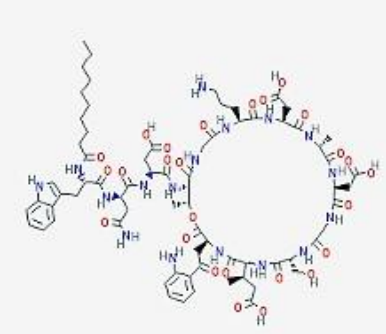
Polymyxin B (Colistin)

The novel antimicrobial peptide based Teixobactin identified [489]. Recently, Scientists have discovered an antibiotic capable of fighting infections that kill hundreds of thousands of people each year, a breakthrough that could lead to the field's first major new drug in more than a quarter-century. The new agent that was isolated, identified and synthesized, gives great hope to medicine regarding the combat withy resistant bacteria. Teixobactin and some of its analogs were synthesized by number of research groups. This awakes the hopes that are centered around the following:

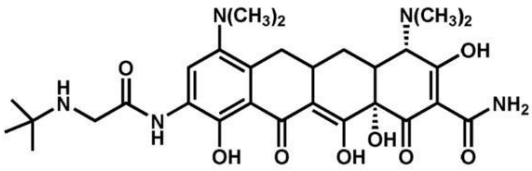
1) Selectivity preferred eradication, Gram+ or Gram-.
2) Effective eradication of all bacteria regular and resistant.
3) Low toxicity to humans.

Not only one target is attacked by Teixobactin but multiple targets, and they are all lethal. For bacteria, it will be very hard to modify this target, especially this part of the molecule that's bound by Teixobactin.

Until the discovery of Teixobactine, the following were the top antibiotics in the clinic:



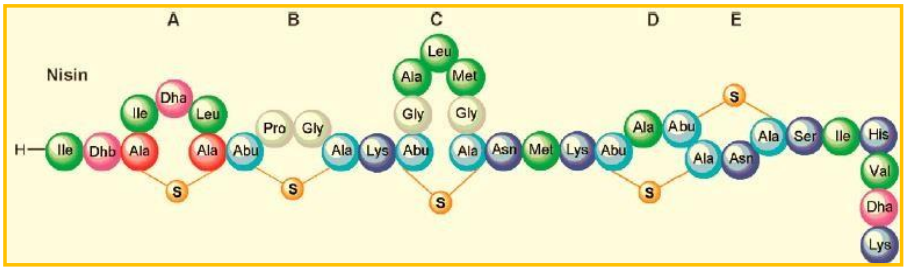
Daptomycin



Tigecycline

The last major new antibiotic, daptomycin, was discovered in the 1980s by Eli Lilly & Co. After being abandoned in early testing, Antibiotic-resistant bacteria kill at least 700,000 people a year, per a U.K. government review. Unchecked, those infections could lead to 10 million more deaths a year by 2050, the report found.

Tigecycline [490] is the one of newest antibiotic to have been released. It has been on the market for years. It this includes activity against gram-positives, gram-negatives except for P. aeruginosa, many anaerobes and many atypical pathogens as well as several resistant pathogens. Because it is not vulnerable to many of the resistance mechanisms that affect the β -lactam antibiotics, it has potential utility in those types of situations. It has been approved for use in complicated skin and skin structure infections, as well as for treatment of complicated intra-abdominal infections due to susceptible pathogens. These are the only 2 indications that are approved right now. It has very interesting pharmacokinetics and a long half-life. It will be dosed, after a load, twice a day, and it has a rather low serum concentration, but very good tissue distribution. Tigecycline is available as an intravenous solution. The primary side effects or adverse reactions that have been seen in clinical trials have been gastrointestinal symptoms, usually nausea.



NISIN

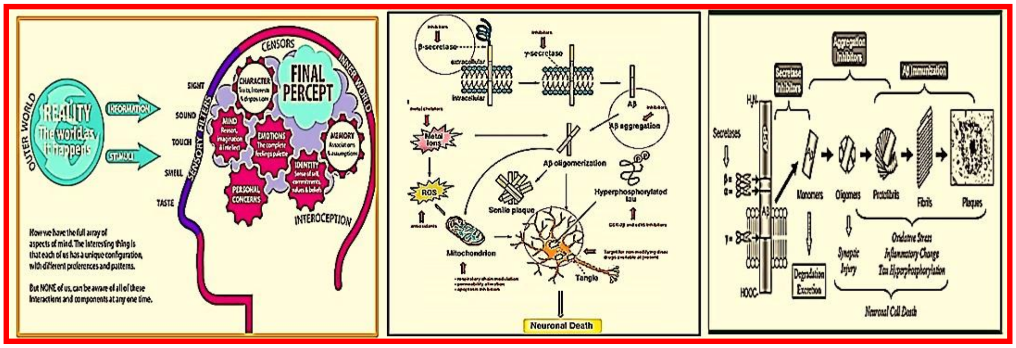
Bioactive Macrocyclic Peptides and Peptide Mimics [491a-c], aib -based peptide backbone as scaffolds for helical peptide mimics, Lantibiotics like Nisin and other peptides and mimics were tested as potent agents.

Peptide Surrogates and Antioxidants in the combat against Neurodegeneration

Abstract

The quest of remedy for neurodegenerative diseases is already more than a century old. It is unclear, applying the moist sophisticated ultramodern methods and equipment, what is in fact going on in the human brain when this curse hits. However, Nowadays the perception is that gut microbiome penetrates the brain via the "Gut-Brain" axis. Either by using nerves or through the bloodstream by moving from a leaky gut to a damaged blood-brain barrier and when in the brain, causing inflammation and subsequent infection, that moves in the brain in a prion-like a mechanism, from one cell to its neighbor until interoception (death) is reached.

The eradication of bacteria bu antimicrobial peptide surrogates in synergy with antioxidants, substances that comes from nutrients, to combat inflammation and the crawling infection might be a proper approach to treat neurodegeneration at its early stages.



The Progression of Neurodegeneration

"The top 10 causes of death, the World Health Organization report [492,493,494,495,496,497,498].

Biology of Ageing and Role of Dietary Antioxidants is a leading avenue of research in the area on nerology. It focuses on oxidative stress that is caused by inflammation as well as infection [499]. *Tea Catechins and Theaflavins*. Tea, next to the water, is the second most popular beverage consumed by humans in the world. Black tea is more widely consumed in Western countries while green tea is preferred in the Eastern world. Black tea extracts mainly contain catechins and theaflavins (Figure). Evidence from clinical trials suggests that the consumption of tea has various health benefits. Scientists demonstrated that drinking either green tea or black tea would lead to a significant increase in plasma antioxidant potential by ferric-reducing antioxidant power (FRAP) assay. Furthermore, it has been reported in different population studies that the consumption of green tea or black tea could significantly reduce DNA oxidation and lipid peroxidation

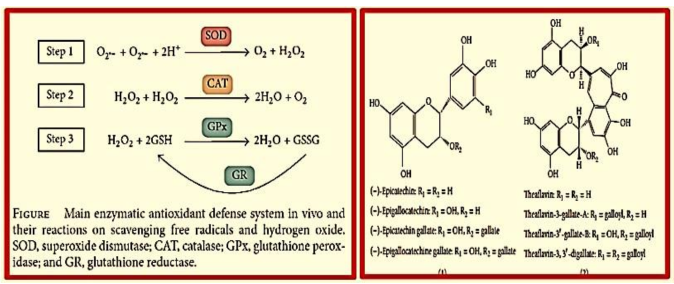


FIGURE Main enzymatic antioxidant defense system in vivo and their reactions on scavenging free radicals and hydrogen oxide. SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; and GR, glutathione reductase.

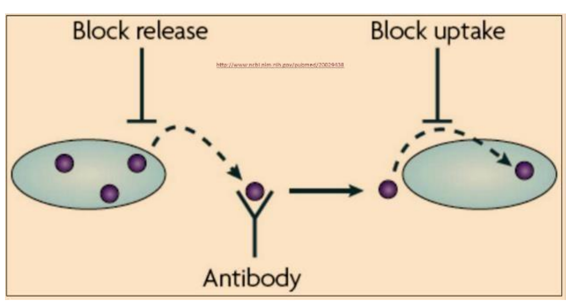
TABLE : Effect of selected nutraceuticals or functional foods on ageing and the possible underlying mechanisms.

Phytochemical antioxidants	Dose	Mean lifespan extension	Molecular mechanism
Apple polyphenols	10 mg/mL	10%	Upregulate SOD1, SOD2, Cat, and Sptll genes. Downregulate MTH gene
Blueberry anthocyanin extract	5 mg/mL	10%	Upregulate SOD1, SOD2, Cat, and Sptll. Downregulate MTH gene
Black rice anthocyanin extract	30 mg/mL	14%	Upregulate SOD1, SOD2, Cat, and Sptll genes. Downregulate MTH gene
Green tea catechin extract	10 mg/mL	16%	Upregulate CuZnSOD, MnSOD, and Cat genes
Black tea theaflavins	5 mg/mL	10%	Increase CAT activity. Upregulate SOD1 and Cat genes
Sesamin	2 mg/mL	12%	Upregulate SOD1, SOD2, and Rpn11 genes.
Curcumin	100 μM	16%	Downregulate the expression of several aging-related genes, including TOR, InR, Thor, mth and rpr
Marine microalga DHA-rich extract	10 mg/mL	10%	Upregulate SOD1 and SOD2 genes. Downregulate MTH gene
Nectarine extract	4%	14–22%	Reduce the transcript level of phosphoenolpyruvate carboxykinase (PEPCK), iron regulatory protein 1B (Irp-1B), 4E-BP. Influence the redox status and reduce oxidative damage indirectly through modulate the JNK signaling pathway.

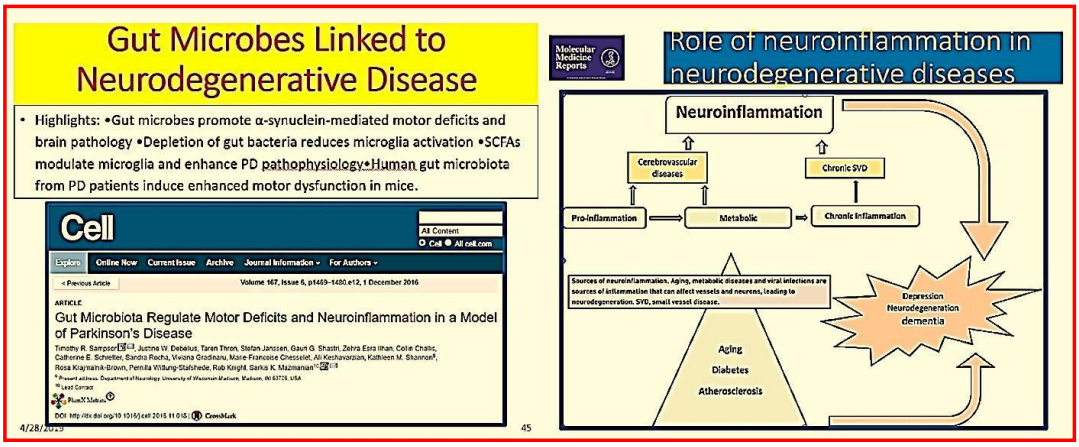
Antioxidants of Green Tea (credit ref. [2]).

Forward

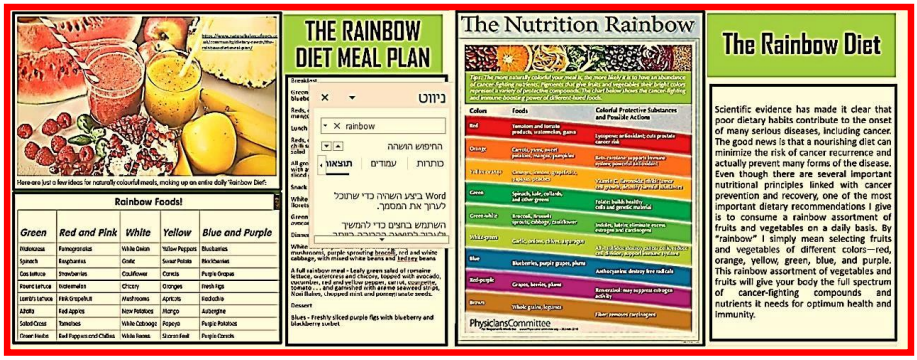
Neurodegenerative disorders such as Alzheimer's disease (AD), stroke, and Parkinson's disease (PD) represent a major clinical problem in the developed countries [500] and are major economic burdens for health care systems. Dietary [501], genetic, and molecular factors [502] are important determinants in the progression and intervention of neurodegenerative diseases. AD is a common cause of dementia and mortality in the United States. Total numbers of reported deaths due to AD have increased in the past years, and it is among 10 leading causes of deaths in the western world [503,504]. Recent work has demonstrated that the microbiota also influences brain function in healthy and diseased individuals. Of great interest are reports that intestinal bacteria play a role in the pathogenic cascade of both Parkinson and Alzheimer diseases. Prion-like mechanisms [505] in neurodegenerative diseases are causing great difficulty in an attempt to stop the spread of the disease in the brain.



If trans-cellular propagation of protein misfolding occurs, then new strategies could supplement existing approaches to promote cell survival and block the intracellular accumulation of misfolded species. As the cellular mechanisms of aggregate release and uptake are delineated, it may be possible to inhibit these events pharmacologically or genetically. Antibody-based therapies might also be expanded to target protein aggregates that are generated inside a cell and released into the extracellular space.

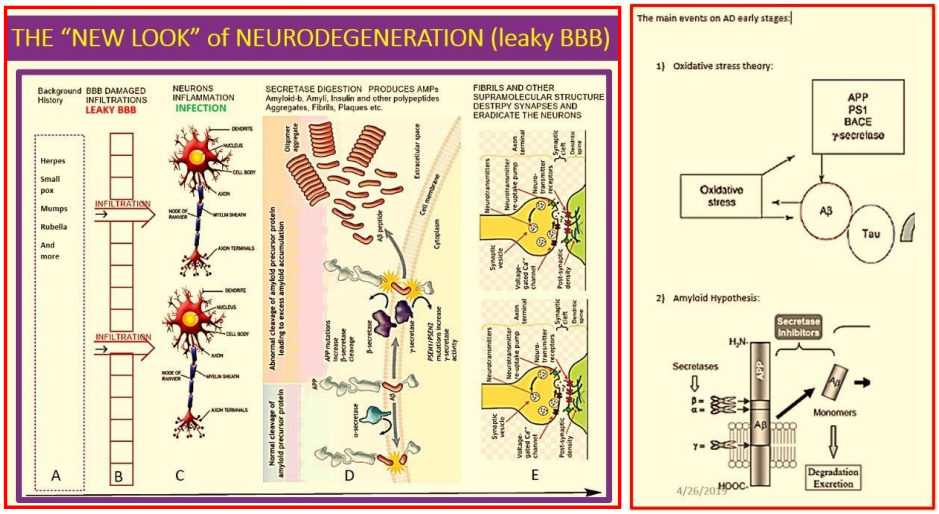


It is believed that "Eating the rainbow"[506,507, 508] is a modern slogan by nutrition experts. This "Mediterranean" food intake is believed by many to bring some remedy to the many millions worldwide that are victims of neurodegenerative diseases, like Alzheimer's, Parkinson's, Brain Stroke and many other. This diet will provide active chemicals produced by nature to combat some factors that initiate and keep the illnesses progressing, slowly but surely. According to a recently applied conception, neurodegeneration may be a cause of penetration of gut microbiome [509] bacteria through a leaky gut and a damaged Blood-Brain Barrier [510-512], infiltrate the barrier and cause inflammation and subsequent crawling infection, in the brain which ultimately brings the patient to death.

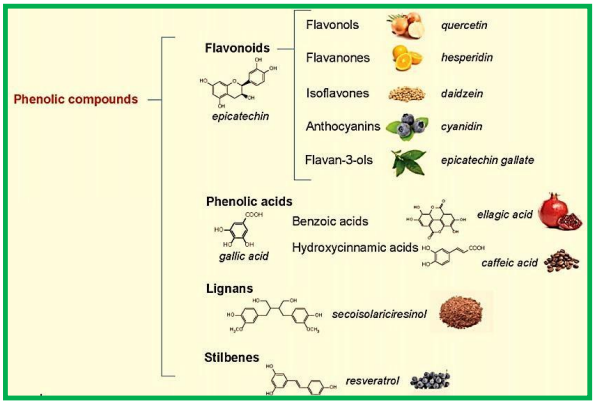


The onset of neurodegeneration is believed to involve oxidative stress due to the invasion of the gut microbiome into the brain tissue which causes inflammation, marked by oxidative stress.

A synergy between antimicrobial and antioxidant agents may address these evolvements. The antimicrobial part maybe based on antimicrobial peptide surrogate and the antioxidant portion may involve polyphenols [513,514] as antioxidants penetrating the brain by nutrition, consuming foods that carry such compounds in its matter.



The gut-brain microbiome axis has received an abundance of attention recently. It is thought that gut microbiota can influence brain function and behavior, but how that happens is still unknown. It has been proposed that bacteria can enter the brain through the blood-brain barrier, and/or via nerves that innervate the gut. Here we show the presence of bacteria in the human and mouse brain under noninfectious or nontraumatic conditions. It was found the bacteria, identified by morphological criteria, in ultrastructural samples of human postmortem brain. Serial section analysis for identification and quantification. All cases contained bacteria in varying amounts. Bacteria were rod-shaped, and contained a capsule, nucleoid, ribosomes and vacuoles. The average diameter of the short axis was 0.496 mm. Many were segmented, with the long axis averaging approximately 1.78 mm between segments. Others did not appear to be segmented and were approximately.



Phenolic compounds in "Mediterranean Diet" (credit ref. 8).

0.866 mm in the long axis. The vast majority of the profiles had a thick capsule of approximately 100nm. The density of the bacteria varied according to the brain region, with abundant bacteria in the substantia nigra, hippocampus and prefrontal cortex but sparse numbers in the striatum. Bacteria were present in intracellular locations, predominantly in astrocytic end feet at the blood-brain barrier, dendrites and the soma of glial cells. They were also abundant adjacent to and within myelinated axons. To address the possibility that the bacteria in human tissue was a result of postmortem artifact, we examined mouse brains that were fixed immediately at death (n=10); there were abundant bacteria in similar intracellular locations. To eliminate the possibility that the presence of bacteria was due to contamination, we examined germ-free mouse brains (n=4) processed in an identical way; we did not detect any bacteria. The observation that the location of the bacteria was highly specific and deep within the specimens also argues against contamination. Interestingly, there were no structural signs of inflammation in any of the brains examined. It is presently unclear the route of entry bacteria take to the brain, but the evidence of them in axons and at the blood-brain barrier supports the previous speculation. α-synuclein (αS) is a nerve cell protein associated with Parkinson disease (PD) [515]. Accumulation of αS within the enteric nervous system (ENS) and its traffic from the gut to the brain are implicated in the pathogenesis and progression of PD. αS has no known function in humans and the reason for its accumulation within the ENS is unknown. Several recent studies conducted in rodents have linked αS to immune cell activation in the central nervous system. We hypothesized that αS in the ENS might play a role in the innate immune defenses of the human gastrointestinal (GI) tract.

Our Brain is Home for Microbiome Dwellers

Treatment of many neuronal degenerative disorders will require delivery of a therapeutic protein to neurons or glial cells across the whole CNS. The presence of the blood-brain barrier hampers the delivery of these proteins from the blood, thus necessitating a new method for delivery.

Antimicrobial peptides (AMPs) are host-encoded antibiotics that combat invading microorganisms. These short, cationic peptides have been implicated in many biological processes, primarily involving innate immunity.

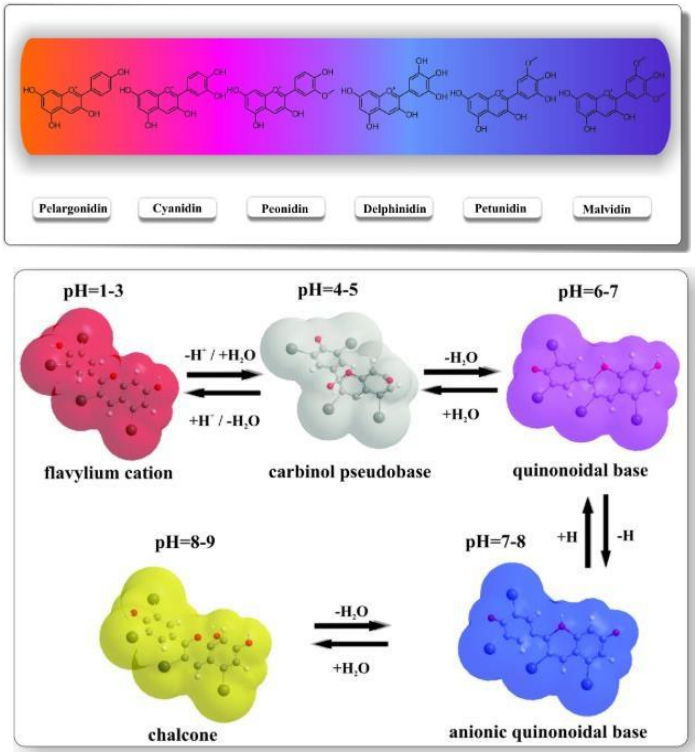
The penetration of microbes into the inner brain can be the onset of inflammation followed by infection in the brain. One can rationalize the digestion of APP and other proteins by enzymes to form antimicrobial peptides like The amyloid b which combat the microbes, but aggregate to form many sorts of solid formations that damage the synapses as depicted above. The series of events that are induced by the oxidative stress of the inflammation, lead to the harming antimicrobial peptides of which the amyloids are only one sort. Since peptides can barely enter into the brain via the BBB [516]. However, it is possible to enter the brain via various receptors, an example is the diazepine receptor [517].

In innate immunity, inflammation-based immunity is the first line of vertebrate defense against micro-organisms. Inflammation relies on a number of cellular and molecular effectors that can strike invading pathogens very shortly after the encounter between inflammatory cells and the intruder, but in a non-specific way. Owing to this non-specific response, inflammation can generate substantial costs for the host if the inflammatory response, and the associated oxygen-based damage, get out of control. This imposes strong selection pressure that acts to optimize two key features of the inflammatory response: the timing of activation and resolution (the process of downregulation of the response).

Nevertheless, host immune regulation also opens the way to pathogens to subvert host defenses.

Currently, many agree that the gut microbiome reaches the brain using thew "gut-brain axis" and is the cause for the onset of neurodegeneration- this penetration results in inflammation, then infection and apoptosis of the brain tissue. Antimicrobial peptide surrogates [518], based on a brain-penetrating scaffold-like diazepine [519] or 1,4 dihydropyridine [520] may be suitable to combat the invading microbiota and in a later stage serve as antimicrobial agents to combat the infection and prevent aggregation.

Blueberries aren't actually blue [521], but deep purple, which is the color of anthocyanin, the pigment that is especially rich in blueberries [523,524]. Blueberries are known to be high in antioxidants, "Blueberries are the King of Antioxidant Foods", which are suitable for the human body; the anthocyanin is thought to be useful for combating inflammation. A good rule to follow is, the darker the berry, the more anthocyanins are present. The main antioxidant compounds in blueberries belong to a family of polyphenols antioxidants called flavonoids.

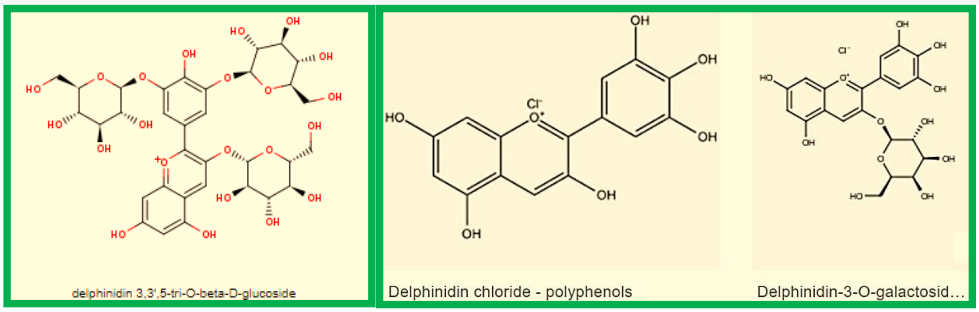


The visible color range of common anthocyanidins (credit ref. [522]).

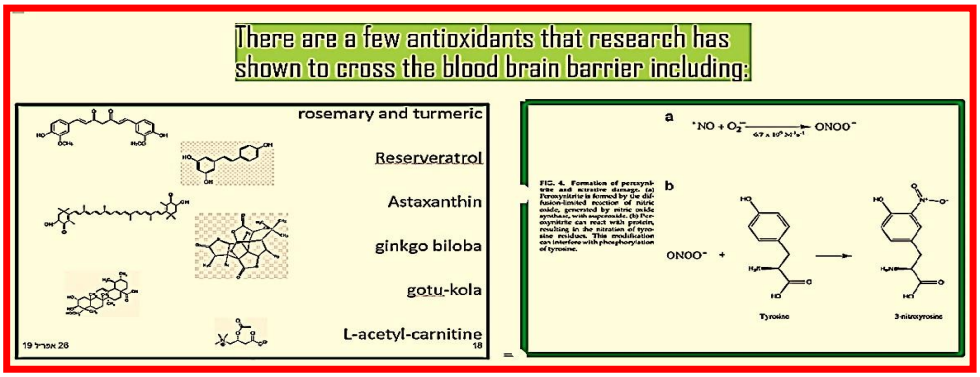
The group of flavonoids in particular- anthocyanins is thought to be responsible for much of these berries' beneficial health effects.

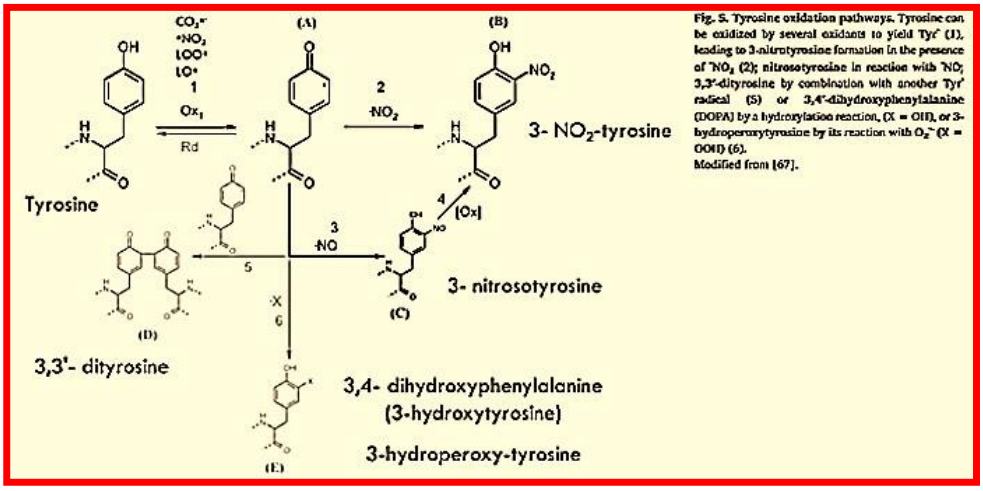
There are many antioxidants that may aid in this circumstance for an instant in a Mediterranean diet or by using forest berries, blueberries [525] that contain many sorts of antioxidants. In fact, blueberry intake acutely improves vascular function in healthy men in a time- and intake-dependent manner. These benefits may be mechanistically linked to the actions of circulating phenolic metabolites on neutrophil NADPH oxidase activities.

Identification of Phenolic Acids, Flavonol Glycosides and Antioxidant Potential in Blueberry, Blackberry, Raspberries and Cranberries were reported [526].



The daily consumption of fruits and vegetables is consistently associated with reductions in the incidence of degenerative diseases, by having antioxidant (The blue pigment Delphinidin for example. Delphinidin is the most common anthocyanidin molecule in blue flowers and forms with pelargonidin and cyanidin the three anthocyanidins known as the principal and basic skeletons of flower color pigments. So they are the most widespread in nature), antiinflammatory and anticarcinogenic activity [527]. Scientists reported [528], that daily consumption of 600 g of fruits or vegetables can prevent the risk of coronary heart disease by more than 31% and ischemic stroke by 19%. Several epidemiological studies summarized in diverse papers [529,530,531,532] have built the consensus that diets rich in fruits and vegetables have beneficial effects on human health.





Antioxidants that are known to cross into the brain and capture of NOX by tyrosine (Credit ref. [30].

Scientists at the University of Basel [533,534] revealed a fundamentally new mechanism explaining the inadequate immune defense against chronic viral infection.

Hope for Remedy?

Crossing the BBB is a significant obstacle in an attempt to find a therapy for neurodegeneration. The brain is protected in many ways, by the bones of the skull, and by many layers of different tissues and finally by the blood-brain barrier, which only selected compounds may cross in both directions using various crossing gates [535].

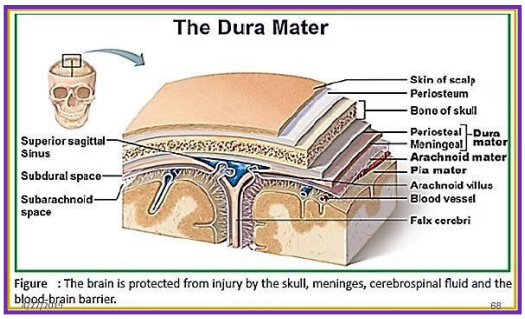
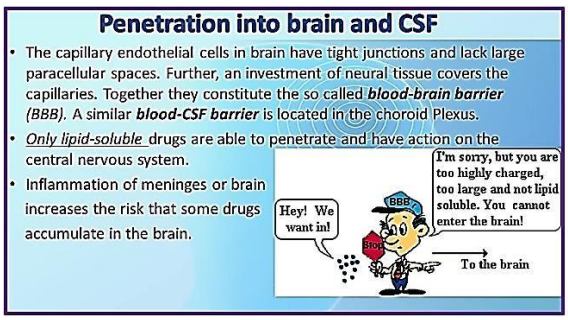
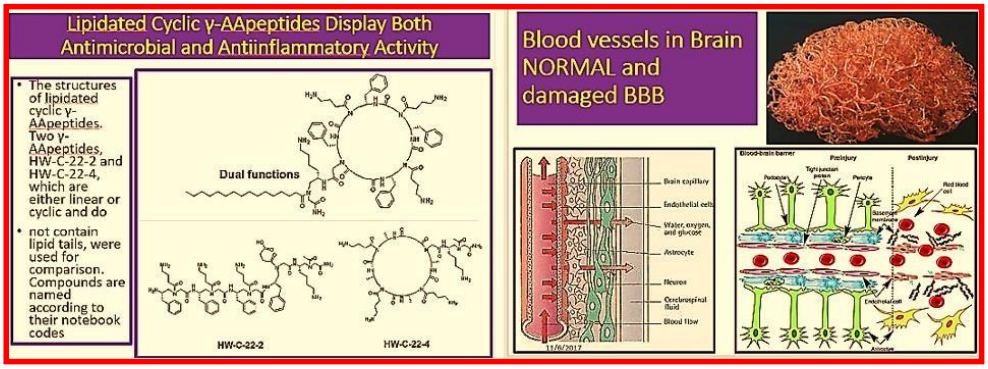


Figure : The brain is protected from injury by the skull, meninges, cerebrospinal fluid and the blood-brain barrier.



The bidirectional crossing of the BBB.

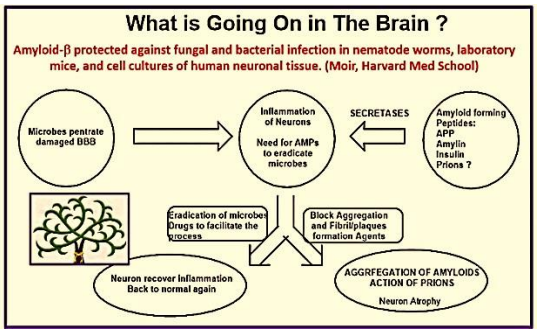
The brain is a protected environment, partially surrounded by the content of the blood stream by a network of cells that surround its blood vessels. Bacteria and viruses that penetrate this blood-brain barrier can cause life-threatening inflammation. Some studies have suggested that distant bacteria - those that live in our intestines - can affect mood and behavior and even the risk of neurological diseases, but indirectly. For example, a disturbance in the microbial balance of the bowel may increase the production of the unruly protein that can cause Parkinson's disease if it moves the nerve that connects the intestines to the brain [536,537].

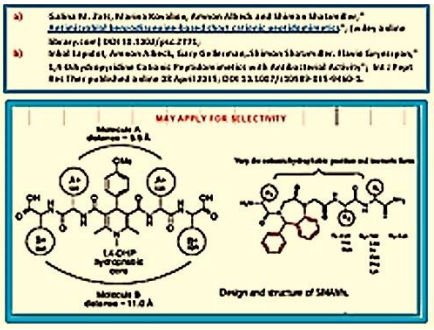


The hallmark brain damage in Parkinson's disease is thought to be the work of a misfolded, rogue protein that spreads from brain cell to brain cell like an infection. Now, researchers have found that the standard form of the protein-α-synuclein (αS)-may actually defend the intestines against invaders by marshaling critical immune cells. But chronic intestinal infections could ultimately cause Parkinson's, the scientists suggest, if αS migrates from overloaded nerves in the gut wall to the brain.

"The gut-brain immune axis seems to be on a cusp of an explosion of new insights, and this work offers a fascinating new hypothesis," says Charles Bevins, an expert in intestinal immunity at the University of California, Davis, who was not involved with the study.

The normal function of αS has long been a mystery. Though the protein is known to accumulate in toxic clumps in the brain and the nerves of the gut wall in patients with Parkinson's disease, no one was sure what it did in healthy people. Noting that a region of the αS molecule behaves similarly to small, microbe-targeting proteins that are part of the body's immune defenses, Michael Zasloff, an immunologist at Georgetown University Medical Center in Washington, D.C., set out to find whether αS, too, might help fend off microbial invaders.

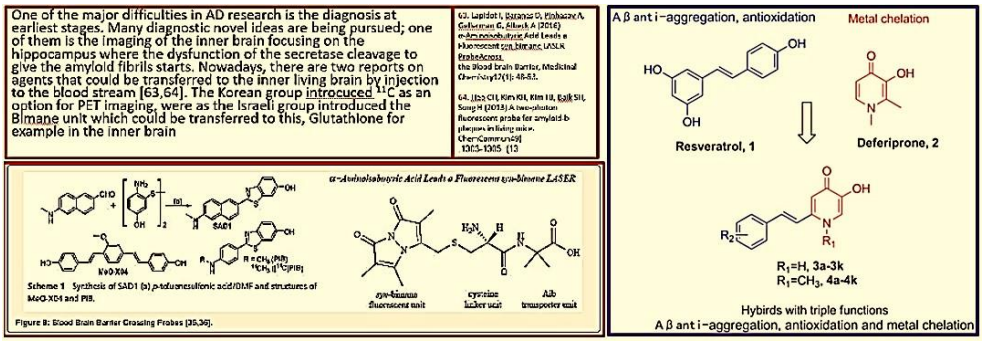




Introducing antimicrobial peptide surrogates to eradicate cerebral microbiome.

Although peptides may enter the BBB into the brain from the bloodstream, in bidirectional traffic [538], antimicrobial peptides may be digested already when in the blood due to intensive enzymatic degradation in the serum. It may therefore be more practical to apply surrogates of such antimicrobial peptides that may contain scaffolds that cross the BBB and thereby enable eradication of the microbial dwellers of the subconscious brain.

Recently [539,540] such mimics were prepared and tested for crossing into the mouse brain via the blood system, the agents were injected into the bloodstream and detected in the inner brain using confocal microscopy. AIB (□-amino iso-butyric acid as a branched-chain amino acid [541]) was used as a "trojan horse" to allow such crossing of the barrier.

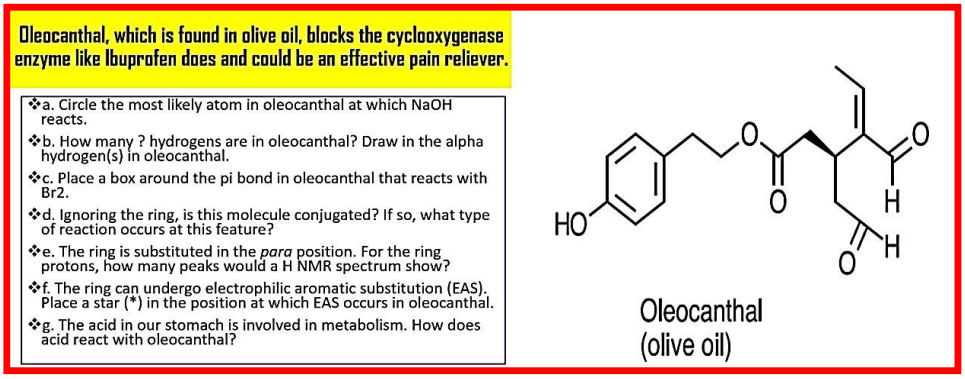


BBB Crossing Agents

Antioxidants

While many lines of evidence indicate high levels of oxidative stress that plays a causal role in a number of neurodegenerative conditions, the current understanding of the specific role of oxidative stress in the genesis and/or spread of neurodegenerative diseases remains well defined. Even more challenging the "oxidation theory of neurodegeneration" is the fact that many antioxidant-based clinical trials and therapeutic interventions have been particularly disappointing to their therapeutic advantage.

It is recommended by physicians and nutritionists to use a "Rainbow" based "Mediterranean diet" as a source of "healthy" antioxidants and vitamins and "Olive Oil [542,543, 544]" which is loaded with bioactive substances. But as a matter of fact, only a few of these can cross the BBB and have a curing effect of neurological dysfunction. Polyphenols are a significant agent to treat neurodegeneration [545].



Benefits of Olive Oil

Physiological Properties of Oleocanthal and Its Putative Health Benefits

Research conducted by Beauchamp and colleagues (Beauchamp et al., 2005) demonstrated that oleocanthal inhibits cyclooxygenase (COX) enzymes in a dose-dependent manner, mimicking the anti-inflammatory action exerted by ibuprofen. Cyclooxygenase 1 and 2 (COX 1 and COX 2) enzymes are responsible for the conversion of arachidonic acid to

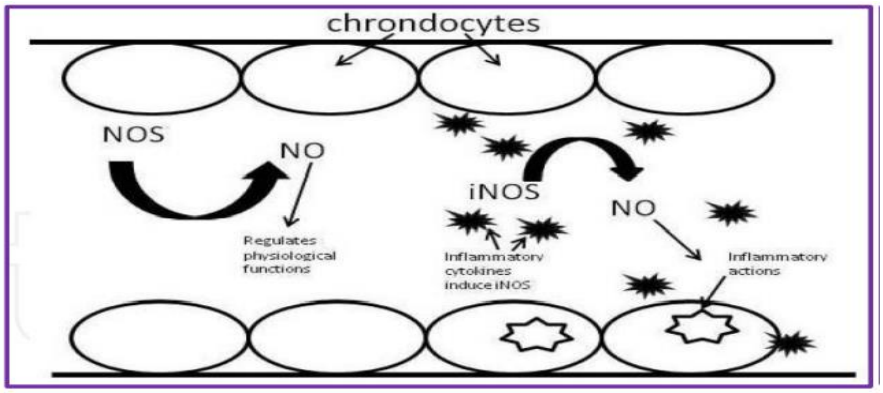


Figure: N nitric oxide (NO) is derived from nitrogen oxide synthesis (NOS), functions as a neurotransmitter and vasodilator, and is essential in normal physiological responses. Nitric oxide nitric synthase (iNOS) is a third form of NOS and is not present in resting cells, but is caused by inflammatory cytokines. NO produced from iNOS promotes inflammation in chondrocytes and is associated with cartilage degeneration.

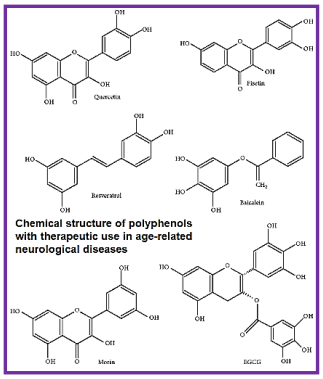
Degenerative, Degenerative, and Joint disease

In vitro studies call attention to Rosenthal as a potential therapeutic

compound that may be interested in seeking natural NSAIDs to treat a common degenerative disease. Pro- inflammatory cytokines arrange the synthesis of cartilage into degrading enzymes and stimulate the production of nitric oxide (Scher et al., 2007), And increase the production of PGE2 prostaglandin, each of which is involved in the development of joint pain and thus joint degeneration disease. COX enzymes are a catalyst for the formation of prostaglandins and have been reported to be highly reflected in the arthritic spine in the animal model.the Therefore, oleocanthal may affect arthritis pain through the inhibition of PGE2 synthesis accompanying COX inhibition. NO plays an integral role in joint degenerative disease and the stable end product of NO, nitrite (NO2), is expressed in a synthetic synthetic fluid significantly. In osteoarthritis (OA) pathogenesis, patient cartilage does not synthesize spontaneously from diseased chondrocytes (Tung et al., 2002). NO is biosynthesized by nitrogen oxide synthesis (NOS). Another form of NOS is the NOS stimulant (iNOS) which is mostly responsible for the inflammatory actions of NOS. Iacano and colleagues (2010) showed that oleocanthal and synthesized derivatives, decreased the production of iNOS protein expression in LPS challenged murine chondrocytes, dependent doses, further highlighting anti-inflammatory actions of oleocanthal and pharmacological potential. Also, as oleocanthal modulates prostaglandin synthesis using inhibitory actions on COX enzymes, it is possible that oleocanthal may trigger pharmacological actions to treat rheumatoid arthritis or osteoarthritis using COX inhibition.

Polyphenols exhibit strong potential to address the etiology of neurological disorders as they attenuate their complex physiology by modulating several therapeutic targets at once. Firstly, we review the advances in the therapeutic role of polyphenols in cell and animal models of AD, PD, MS, and HD and activation of drug targets for controlling pathological manifestations. Secondly, we present principle pathways in which polyphenol intake translates into therapeutic outcomes. In particular, signaling pathways like PPAR, Nrf2, STAT, HIF, and MAPK along with modulation of immune response by polyphenols are discussed.

Although current polyphenol researches have limited impact on clinical practice, they have strong evidence and testable hypothesis to contribute clinical advances and drug discovery towards age-related neurological disorders.



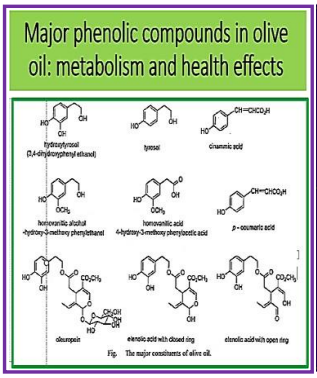


Table 1:Neuroprotective signal transduction by polyphenols.

Pathway	Polyphenol
P13K/AKT pathway	Resveratrol
	Baicalein
NFκB pathway	Kaempferol, acacetin, apigenin, luteolin
	Soybean isoflavones
	Fisetin
	Resveratrol
	Baicalein
	Silymarin
	Tetrahydroxystilbene
	Quercetin
	Catechin hydrate
STAT pathway	Silymarin
PPAR pathway	Baicalein
	Resveratrol
	Pterostilbene
Nrf2/HO1/ARE pathway	Epicatechin
	Resveratrol
HIF-1α	Xanthohumol
	Resveratrol
MAPK	Flavone glycoside
	Quercetin

Polyphenols also protect mitochondria from pathological events by triggering presurgical cell signaling. Polyphenols increase antioxidant enzymes, that is, catalase, superoxide dismutase (SOD1, SOD2), and PR survival Bcl-2 and ERK pathways. Downregulation of Bad/Bax, c-jun, JNK, COX-2, AP-1, and caspase-3 also contributes to the survival of neurons. Polyphenols also help in improving cognitive abilities by inhibiting AChE and BChE. The inhibition of these enzymes plays an essential role in clinical medicine of AD. Apart from their anti-AChE activity, polyphenols also induce metal chelation and modulate autophagy and prion proteins. These features along with the reduction of Aβ toxicity, reduction of neural lesions, and activation of cell survival genes are of particular relevance to neurodegenerative diseases. The activation of the novel spectrum of these molecular targets forms the underlying mechanism of neuroprotection by polyphenols. The lack of toxic effect and availability from natural sources makes polyphenols as clinically relevant therapeutics in neurodegeneration. Synergic effects in the quest for remedy suitable for neurodegeneration will include antimicrobial peptides and antioxidants to cure inflammation and infection [546,547].

Conclusion

Studies have shown that neuron cells are particularly vulnerable to oxidative damage due to their high unsaturated fatty acid content in membranes, high oxygen consumption and weak antioxidant protection. However, the exact molecular pathogenesis of neurodegeneration associated with the disruption of the acidification balance remains unclear. New antioxidants have shown great potential in mediating disease phenotypes and can be an area of interest for further research. We provide an up-to-date discussion on the roles of ROS in the pathological mechanisms of Alzheimer's disease, Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, and the ataxia hypoxapler, and an emphasis on antioxidant-based drugs to alleviate the severity of the disease.

Use of Phages in Combination with Antibiotics

The marked increase in the incidence of infections due to antibiotic-resistant gram-negative bacilli in recent years is of great concern, as patients infected by those isolates might initially receive antibiotics that are inactive against the responsible pathogens. However, "When there is a will there is a way". Phage therapy combined with anti-infective agent's synergy [548] might show

the way of combat with the multidrug-resistant microbes.

Abstract

Bacterial infections resistant to antibiotics are a major public health concern. Phage therapy has been suggested as a promising alternative to antibiotics, but a growing number of studies indicate that both microbial agents in combination are more effective in controlling pathogenic bacteria than on individuals. We support the use of phages in combination with antibiotics and present the evolutionary basis for our claim. We also recognize the compelling challenges to the real application of page-antibiotic therapy [549].

Introduction

The absence of production and introduction of new and more effective antibiotic / antibacterial drugs in clinical practice during the post-antibiotic gold era has increased the appearance of resistant pathogenic bacterial infections that pose a significant problem in global human health. The current situation is that in 2011, about 722,000 patients had an infection while in hospital for acute treatment in the US, 205 Americans die of infections acquired in hospital (acquired infections in HAI hospital, nosocomial infections) daily.

Microbial is the immediate threat of reducing detection and development of new antibiotics [550,551,552,553].
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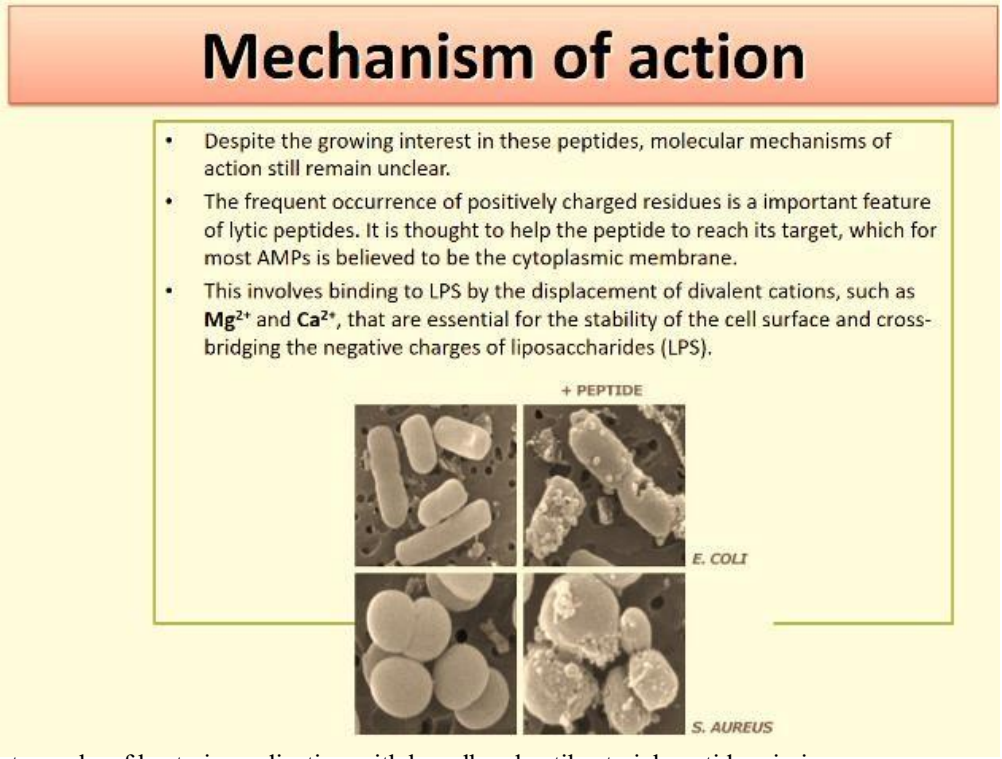
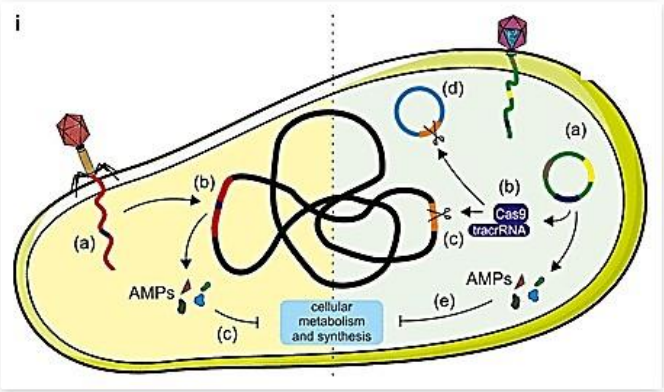


Figure: SEM photographs of bacteria eradication with broadband antibacterial peptide mimics.

Wall teichoic acids are found only in certain Gram-positive bacteria (such as staphylococci, streptococci, lactobacilli, and Bacillus spp); so far, they have not been found in gram- negative organisms. Teichoic acids are polyol phosphate polymers, with either ribitol or glycerol linked by phosphodiester bonds; their structures are illustrated in Figure 2-9. Substituent groups on the polyol chains can include D-alanine (ester linked), N- acetylglucosamine, N-acetylgalactosamine, glucose; The alternative is typical of a particular bacterial species and can be used as a specific antigenic agent. Tychonic acids are covalently linked to peptidoglycan. These highly negatively charged polymers of the bacterial wall can be used for cation-sequestering [554].



Figure

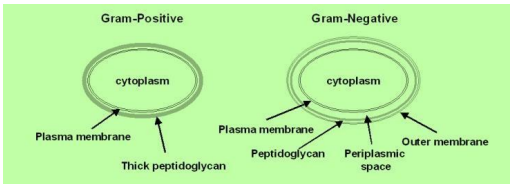
Overview of engineered nonlytic antibacterial phage technologies.
(i) Temperate phage engineered to deliver synthetic gene network (blue) (a), undergo a latent lifecycle after infection, called lysogeny. Here, the viral genome (red) integrates into the bacterium's chromosome as a prophage (b) where it can express antimicrobial proteins (AMPs) that interfere with intracellular processes and cause bacterial death (c). (ii) Phagemids can also deliver synthetic gene network(s) (blue) on a synthetic plasmid (a) that encode for antibacterial proteins, such encoding a RNA-guided CRISPR-associated (Cas) nucleases (b) for sequence-specific (orange) nonlytic bacterial death (c) and plasmid removal (d). Phagemid plasmids can also encode for AMPs (e).

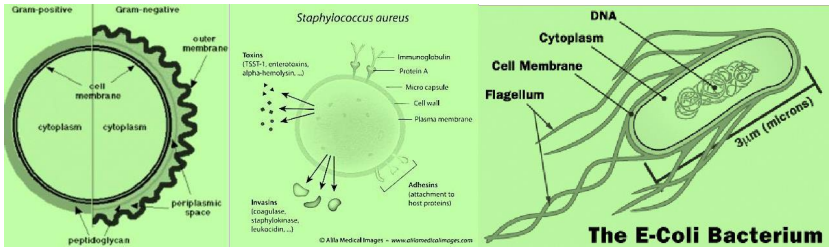
Natural phage therapies rely on causing bacterial death through rupturing cells. However, rapid bacterial lysis might result in the release of endotoxin and inflammatory mediators into the surrounding environment with adverse effects. In contrast, phages can be engineered to be bacteriostatic by deleting genes responsible for lysis (e.g. endolysin) Some tailed phages, referred to as 'temperate', can undergo a latent lifecycle after infection, called lysogeny, and deliver synthetic gene networks with desirable nonlytic antimicrobial properties (Figure). Here, the viral genome either integrates into the bacterium's chromosome as a prophage or remains free in the cytoplasm, which is then replicated alongside the bacterium until conditions favor reactivation to produce virions. Although temperate phages are generally avoided in natural phage therapies, they have been used to deliver synthetic gene networks that can disrupt cell–cell communication between bacteria involved in biofilm formation or to work as adjuvants to antibiotics, such as by repressing DNA repair mechanisms or overexpressing sensitizing proteins. The drawback of these approaches is that the temperate phages would be inherently nonlethal, which one can argue would unnecessarily complicate treatment over using bacteriolytic phages. Prophage-encoded genes also carry the risk of providing a variety of benefits for their bacterial hosts. These beneficial genes are often contained within 'moron' elements and suggest that temperate phages have a symbiotic relationship with bacteria rather than being purely parasites as virulent (lytic) phages [555].

Patients are infected annually, and Institute for Healthcare Improvement (IHI) estimates that more than 5,000 patients die each year as a result. While most patients are treated successfully, particularly if the infection is identified early, hospital stays are often extended by an average 9.1 days, accounting for excess costs of about $20,000 per patient. The total cost burden to the US health care system from MRSA infections is estimated at more than $2.5 billion annually.

Gram-positive and Gram-negative bacteria exist everywhere (a typical post-surgery infection in blue table below) [556] but pose unique threats to hospitalized patients with weak immune systems. Gram-positive bacteria cause tremendous problems and are the focus of many eradication efforts, but meanwhile, Gram-negative bacteria [557] have been developing dangerous resistance and are therefore classified by the American Centers for Disease Control and Prevention (CDC) as a more serious threat. For this reason, the need for modern technologies that kill bacteria, both Gram-positive and Gram-negative, are essential to make hospitals safer for everyone. Both kinds of bacteria may cause fatal infection and should be eradicated one in the presence of the other.

The treatment of those serious bacterial infections in clinical practice is often complicated by antibiotic resistance. Therefore, there is an urgent need for innovative ideas in design and application of antimicrobial agents since bacteria gram-negative bacteria [558,559], develop strains that are intrinsically resistant to many antibiotics (persister) [560] strains, that are practically indifferent to all known antibiotic drugs. Furthermore, in the last decades, only two antibiotic classes with a novel mechanism of action (example is Teixobactin [561,562], a new cell wall inhibitor) have been marketed, and none of them are effective against persister Gram-negative bacteria. (The Top Ten Most Dangerous Bacteria on Earth [563]).





Gram-positive | Ex. *Streptococcus*
Thick peptidoglycan layer absorbs surrouding materials, even toxins. Easier to kill, develops resistance slower.

Gram-negative | Ex. *E. coli*
Thin peptidoglycan layer covered by multiple thin layers of membrane which eject toxins. Harder to kill, quick to develop resistance.

To date, most antibiotics are targeted at intracellular processes and must be able to penetrate.

Discussion

The misuse of antibiotics has reduced their efficacy in controlling pathogens and has led to an increase in the number of antibiotic-resistant bacteria. As an alternative to antibiotics, bacteriophages have become a topic of interest with the emergence of multidrug-resistant bacteria, which are a threat to public health. Recent studies have indicated that bacteriophages can be used indirectly to detect pathogenic bacteria or directly as biocontrol agents. Moreover, they can be used to develop new molecules for clinical applications, vaccine production, drug design, and in the nanomedicine field via phage display.

The Use of phages in as anti-infective in therapy can be extremely successful based on the selectivity of the bacteriophages toward bacteria. Also, the mechanism of action directed at the genetic materials in the nuclei of the microbial cells is different from the cell wall disruption characteristic to most antibacterial agents, mainly antimicrobial peptides and their surrogates. However, Hospital-acquired infections are; like post-surgical infections are characterized by their multi-infectors blend.

We can see more than five infectors in the post-surgical infection in the Ethiopian clinic. Here we can find many sorts of infectors, Gram-negative like Klebsiella spp. And Gram- positive S. aureus, treatment of such wounds will apply a mixture of phages, whereas today's treatment can apply one broad band anti-infective agents (delivered as a pill, injection, infusion) for all microbes listed [564].

Frequency of aerobic bacteria from postoperative wound infection at Ayder Teaching and Referral Hospital, Mekelle, Ethiopia (January to June 2012)

No.	Gram	Microorganism	Frequency N (%
1	G+	*S. aureus*	40 (34.2)
2	G-	*Klebsiella* spp.	29 (24.8)
3	G+	CoNS[a]	18 (15.4)
4	G-	*Proteus spp.*	15 (12.8)
5	G-	*P. aeruginosa*	11 (9.4)
6	G-	*E. coli*	6 (5.1)
7		*Citrobacter* spp.	4 (3.4)
		Total	123 (100)

Table: List of infector microbes found in a post-surgical infected wound. Frequency of aerobic bacteria from post-operative wound infection at Ayder Teaching and Referral Hospital, Mekelle, Ethiopia (January to June 2012) [565].

The past few years have seen a significant resurgent interest in the old concept of using phage as a therapeutic tool. No doubt reports of declines both in antibiotic efficacy and in pharmaceutical company interest in developing new agents are fueling the drive for antibiotic alternatives. Although they are not without their constructive critics, whole-phage approaches may certainly become valuable anti-infective tools in certain therapeutic applications. That said, we and others envision an alternative use for phage that, although less heralded [566], focuses on their specific antibacterial components, rather than the infective virion, as the active killing agent. With the lysins emerging as the most promising antibacterial candidate, defined by their potent and specific lytic activities, we are proceeding with lysin development. Whether used topically or systemically, in humans or agriculture, as purified proteins or as forms expressed from transgenic animals or bacterial secretory systems, an increasing body of data validates the potential utility of lysins. Even beyond the lysins, phage is professional parasites of bacteria, and as such, employ an arsenal of agents to subvert host function and structure. It is only logical that a comprehensive search for new antibacterial agents would attempt to mine this vast viral pool of antibacterial functions. If it is at all possible that the 'parts are greater than the sum' for phage, this work is justified [567].

Indian researchers published on the test of bacteriophage versus antimicrobial agents for the treatment of murine burn treatment [568]. The widespread use of antimicrobial agents in hospital settings has led to the emergence of multidrug-resistant organisms of low virulence such as Klebsiella causing serious opportunistic infections. Beside this, concerns about problems such as high cost of treatment and inability to restore initial appearance of skin have resulted in research on newer agents for the treatment of burn wounds.

Bacteriophages or simply phages can be the best answer to antibiotic resistance in the treatment of bacterial infections [569]. These phages are economical, safe, self-replicating and effective bactericidal agents. In our earlier studies, we have reported the efficacy of phage therapy in treating various infections when injected systemically. In the study, the efficacy of topical application of silver nitrate and gentamicin was evaluated and compared with that of a well-characterized Klebsiella-specific phage, Kpn5, for treating K. pneumoniae B5055 induced burn wound infection in BALB/c mice.

Phage isolation: Klebsiella-specific phage Kpn5 was isolated from a sewage sample. Its utility in treating K. pneumoniae B5055 induced burn wound infections on i.p. injection has been established (Kumari et al., 2009). In this study, phage Kpn5 was evaluated for topical treatment of burn wound infection. Hydrogel preparation is also possible as ointment to treat wounds. There are approaches used in the published literature on the formulation and stabilisation of phage for storage and encapsulation of bacteriophage in micro- and nanostructured materials using freeze drying (lyophilization), spray drying, in emulsions e.g. ointments, polymeric microparticles, nanoparticles and liposomes. As phage therapy moves forward towards Phase III clinical trials, Researchers are looking at promising new approaches for micro- and nanoencapsulation of phages and how these may address gaps in the field. [570].

This study highlights the importance of silver nitrate, gentamicin and phage Kpn5 for controlling burn wound infections caused by nosocomial pathogens such as K. pneumoniae. A single application of phage Kpn5 was found to be superior to multiple applications of silver and gentamicin in the treatment of burn wound infection caused by K. pneumoniae B5055 in BALB/c mice. Studies using these compounds in combination to treat burn wound infection are warranted. This will not only increase the survival of the infected animals, but such a strategy will also keep a check on the development of resistant mutants, a problem frequently encountered by clinicians. In a recent study, we have demonstrated such an effect on treating biofilms of K. pneumoniae B5055 with a combination of ciprofloxacin and phage [571].

Reports [572] indicate that appropriate administration of living phages can be used to treat lethal infectious diseases caused by gram-negative bacteria, such as Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumoniae, Vibrio vulnificus, and Salmonella spp., and gram-positive bacteria, such as Enterococcus faecium and Staphylococcus aureus. The phage display system and genetically modified nonreplicating phages are also effective for treatment of Helicobacter pylori and P. aeruginosa, respectively. In addition to phage particles per se, purified phage-encoded peptidoglycan hydrolase (lysin) is also reported to be effective for the treatment of bacterial infectious diseases caused by gram-positive bacteria such as Streptococcus pyogenes, S. pneumoniae, Bacillus anthracis, and group B streptococci. All phage lysins that have been studied to date exhibit immediate and strong bacteriolytic activity when applied exogenously. Furthermore, phage coded inhibitors of peptidoglycan synthesis (protein antibiotics), search methods for novel antibacterial agents using phage genome informatics, and vaccines utilizing phages or their products are being developed. Phage therapy will compensate for unavoidable complications of chemotherapy such as the appearance of multidrug resistance or substituted microbes.

However successful, Phage therapy seems to be extremely efficient in infections that are a result of one resistant type of infector. However, in cases where infections are caused by many microbes, the therapeutic efficacy of bacteriophage and the antibiotic Agent (A pharmaceutical substance) individually and in combination [573] to treat coli bacilli may be more effective.

Another clinical trial was conducted to evaluate a combination of the antibiotic enrofloxacin and intramuscularly administered

bacteriophage. Both treatments individually provided effective treatments of the E. coli infection, but the synergy between the two treatments led to the total protection of the birds, thus suggesting a significant value of the combined treatment [574].

Key to any successful drug development is its discovery and subsequent characterization. For phage therapy, equivalent steps should be taken, including determination of how to combine [575].

Phage isolation is typically done in combination with preliminary host-range characterization, i.e., as regarding enrichment and isolation hosts. This is followed by in vitro characterization in association with further host-range characterization (i.e., involving a larger panel of potential hosts) and bioinformatic (in silico) characterization. Enzybiotic development, if undertaken, typically will follow host-range and in silico characterization.

For promising phages, in situ characterization comes next, including animal models for potential human treatments (in vivo characterization), or with other species for non-human treatments. Clinical testing can follow, including treatment of non-human species.

Alternatively, phages may be employed for biological control of environments, and both biological control and therapeutic use of phages can be against biofilms. Not only may whole phages be used for therapy or control but so too may enzybiotics. Further development toward successful commercial or public-sector implementation generally must address regulatory requirements
Phages into multi-phage mixtures known as phage cocktails. The review article in this topic by Weber-Da˛browska et al. discusses the essential steps involved including sources and the Methods of phage insulation, selection of flower distribution host, characterization methods, selection criteria for therapeutic purposes, and limitations on phage procurement for treatment. The use of phages as antibacterial therapy is especially important for targeting these pathogens, which have limited antibiotic treatment options. Isolation on demand of the corresponding phages can be achieved through enrichment of samples from environmental reservoirs, as investigated by Mattila et al. Interestingly, the differences between phage isolation based on enrichment and urban wastewater vary considerably, with the best results for, Salmonella, and the complex β-lactamase spectrum (ESBL) producing Escherichia coli and Klebsiella pneumonia. The procedure is less useful for vancomycin-resistant to vancomycin and Acinetobacter baumannii, while the isolation of new phages against resistance to methicillin Staphylococcus aureus (MRSA) strains was highly ffi cult. Eventually, the latter may be due to the selection of an environmental buffer used for isolation against MRSA phage since, as Wang et al. To show, .pig swine flu may be a better source for these bacteriophages.

Concluding Remarks Overuse of Antibiotic Drugs

Abstract

The widespread use of antibiotics in the past 80 years has saved millions of lives, facilitated technological advances and killed unprecedented numbers of bacteria, pathogens and pigs. Human-related bacteria perform a series of important functions, and now we are beginning to understand how antibiotics have reshaped the ecology and the functional implications of these changes. Findings show that antibiotics affect the function of the immune system, our ability to resist pollution, and our ability to process food. Therefore, it is now more important than ever to examine how we use antibiotics

This article summarizes the current study on the short-term and long-term effects of antibiotic use on the human microbiota, from early life to adulthood, and its impact on diseases such as malnutrition, obesity, diabetes, and Clostridium difficile infection. Driven by the results of incorrect use of antibiotics, we are investigating the latest advances in the development of anti-virulent approaches to infection resistance while minimizing treatment resistance. We conclude the article by discussing probiotics and transplants in microbiota feces, which promise to return the microbiota after the damage of the microbial. Together, the results of studies in this area emphasize the importance of developing a mechanistic understanding of intestinal ecology to enable the development of new therapeutic strategies and to limit the rational use of antibiotics.

Foreword

Over the past decade, our knowledge of the role of gut microbial in health and disease has increased dramatically, accompanied by an invisible hype surrounding its diagnostic and therapeutic potential. However, one area of application of the microbiome has so far remained distinct: its role as treatment instruction.

Microbium research and improvements in high-output sequencing technologies revolutionized our current scientific point of view. The associated human microbiology is a prominent focus of clinical research. Large studies are often needed to study the composition of human microbial and the changes that have occurred in many human diseases.

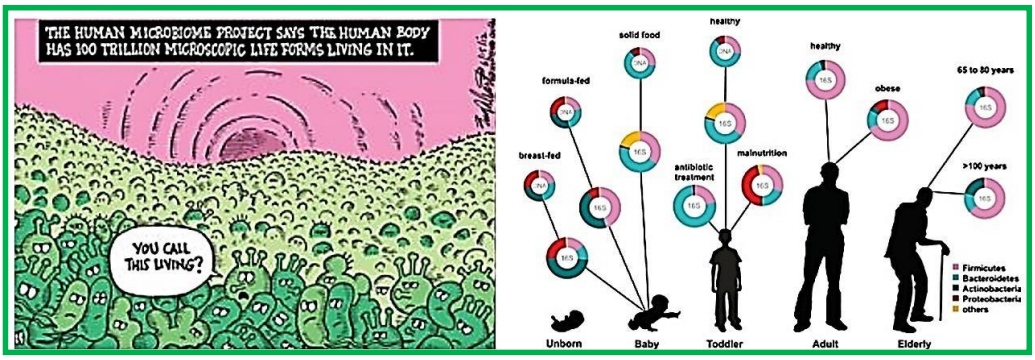
The average longevity of a US citizen born in 1940 was expected to live to age 63. A baby born today should reach 78, partly because of antibiotics, but the assumption that safe antibiotics generally fostered overuse and led to increased bacterial resistance to treatment. Other, no less dangerous, of our love for antibiotics have received more attention.An antibiotic kills the bacteria we want as well as those we do not.Interior evidence from the laboratory and other deceptions, sometimes, our thick vegetation never recovers. This long-term changes in beneficial bacteria In people's bodies may even increase our susceptibility to infections and stress Overuse of antibiotics may fuel the dramatic increase in conditions such as obesity, diabetes, inflammatory bowel disease, allergies and asthma, which have doubled more than many populations.

Diabetes type 2 and Obesity are considered by many to be behavioral diseases; "The patient eats too much"-eat less….. The more modern perception links this with our symbiotic partner in life: the microbiome. Alternation of the population of microbes

that lives within us throughout our entire life. It is not clear how these changes occur and more importantly, how to harness the billions of organisms to avoid these terrifying series and save the agony that comes with them. The overuse of Antibiotic medicines can become a harming cause that facilitates the changes in the constitution of the microbiome.

Global Human Microbiome Market: Venture Capitalists See Promise in Microbiome-based Technologies. Only to Increase Investments [576]. The following are general targets for microbiome R&D: Gastrointestinal Disorders, Metabolic Disorders, Women's Health,Skin Disorders. Main technologies applied are in the area of genome sequencing: 16s rRNA Sequencing and Metagenomic Sequencing [577].

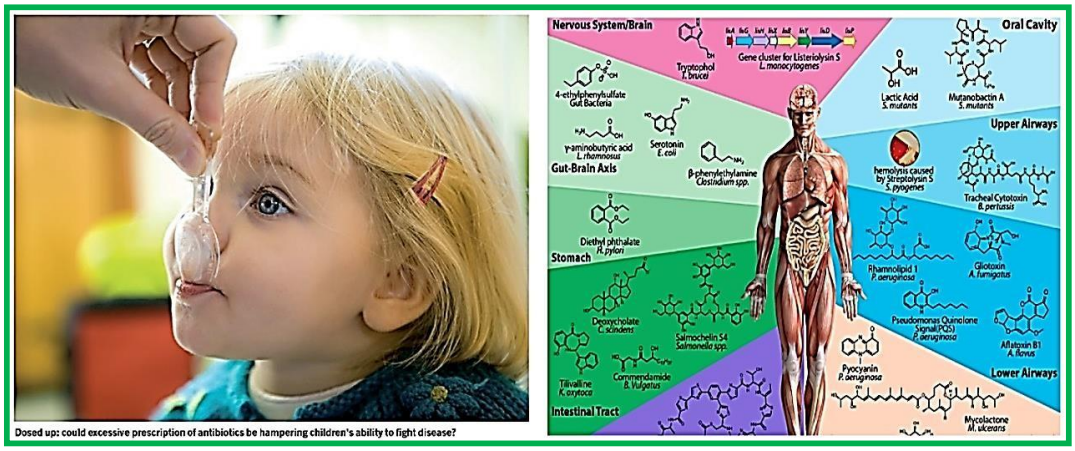
Over the past 15 years [578], The invisible microbial world is aided by the center of the stage thanks to DNA sequencing methods that allow researchers to identify bacteria and other organisms that can not be cultured in culture, first of all, found large and diverse communities within our intestines, our skin, Later, studies involving mice without germs and other studies have revealed links between these bacteria, so-called microbiota, and health-with bacteria that play key roles in immunity, obesity and development, so much happened that both 2011 and 2013 Science called microbiome as one of its breakthroughs of the year in 2012 and 2016. Today, the meter the goal is to cover the progress that exposes the specific ways in which the microbiota influences the physiology of the host, both healthy and ill, and how microbial, organically or molecularly, can be treated to improve host health. And recognize that viruses also have an impact, and understand how specific bacteria and their products contribute to healthy and sick countries.



Global Human Microbiome Market Size, Status and Forecast 2025 presents an in-depth assessment of the Human Microbiome including enabling technologies, key trends, market drivers, challenges, standardization, regulatory landscape, deployment models, operator case studies, opportunities, future roadmap, value chain, ecosystem player profiles and strategies. The report also presents forecasts for Human Microbiome investments from 2018 till 2025.

The microbiome [579] is specialized to a specific gut habitat, species within the genus Helicobacter are remarkably specific for particular hosts [580]. For example, H. pylori are associated with humans [581], and *H. hepaticus* is associated with mice. Gastric Helicobacter species are autochthonous: They have been (until the antibiotic age) nearly universally prevalent and usually not pathogenic to their natural host.

Another example of a highly host-adapted gut microbe is L. reuteri [582]. Many "small molecules" and peptides are associated with the microbiome [583].



The microbiome, our symbiotic partner for life, seems to influence our physical situation in a healthy manner. Since the emergence of simple automatic gene sequencing technologies, the application of those for therapeutic targets are emerging rapidly. Those many illnesses like Rheumatism, Obesity, Diabetes, Neurodegeneration and mental disorders are targeted as subjects for this effort.

It seems that the microbiome constitution is altered by the application of therapeutic agents, mainly antibiotics that are so common in today's therapy. However beneficial, some of these agents are damaging. The future effort in antibacterial research shout considers microbiome sensitivity as well.

The Gut Microbiota: A Structural Overview

The microbial and viral communities found in human fecal samples are relatively stable over time and remarkably resistant to blooms of subpopulations, dietary changes, and antibiotics in moderate doses. These findings indicate that the microbial communities present in the large intestine (LI) are to a major degree dominated by an inhabitant of a place (autochthonous) microbes.

Human microbiota consists of 100 trillion bacteria belonging to several hundred different species. These fall into four main groups covering more than 90% of the bacterial population, namely pyramids, bacterias, actinobacteria and proteobacteria, and include many other minor cornerstones such as Verrucomicrobia and Fusobacteria [584]. The representation of these groups varies throughout the gastrointestinal tract (GI) [585], which is affected by certain microorganisms and nutrient feedings. The lawsuit is based on bony, aerobic, and verbal bacteria. Prominent members are Clostridia strains, whose activity ranges from beneficial shields (eg, C. scindens, IV-XIVA clusters) to pathogenic (eg, C. difficile, C. perfrigens). Streptococcus pathogens, enterococci, and staphylococci are also firms. Bacteroidetes are Gram - bacteria that are well adapted to the intestinal environment. Here they ferment carbs that are indigestible, producing SCFA, molecules that have been involved in a multitude of important processes. Actinobacteria are gram + bacteria and are generally considered beneficial, such as the Bifidobacterium genus, and which are included in many probiotic preparations. Proteobacteria Shelter contains Gram-bacteria, especially the family of Enterobacteriaceae, including E. coli and K. pneumonia. These are not abundant under normal conditions, but tend to expand on dysbiosis [586]. Most studies of microbiota have been performed in mice, although the microbiota of the individual and the mouse are different in the genotype. Some types of genes such as pravotella, paklibacterium and romanococcus are abundant in humans, while others, namely lactobacillus, alistipus and torsiabacter, are widespread in mice [587]. However, it is possible to identify the core of a common task, and mice and human metagnomes look remarkably similar if analyzed from a functional point of view [ie, the representation of the encyclopedia of genes and the genome of Kyoto, describing the overall metabolic potential of a community [588]. More importantly, the growth factor (GF) [589] can be effectively restored with bacterial communities isolated from other species, including humans, salary effects that were observed in turn in the recipient's host. Reconstruction of GF mice with stool samples of oils or subjects is malnourished enough to phenocopy patient defects in energy harvesting or growth [590], demonstrating that despite interstellar differences, the mouse model is a valuable tool to study the human microbite.

Antibacterial agents are very effective in killing bacteria. However, there is considerably disputable surrounding their health benefits. Materials that do not produce residues (Table of Antibacterial) are used for a long time and continue to be active agents for controlling disease organisms in a wide range of local health services. When used under strict guidelines of application, residues in manufacturing agents have proven effective in controlling bacterial and fungal infections and clinical settings such as hospitals, nursing homes, nurseries and other health institutions where there may be a high risk of infection.

The microbial and viral communities found in human feces are relatively stable over time [591] and are remarkably resistant to the growth of subpopulations, dietary changes [592], and antibiotics in moderate doses [593]. These findings indicate that the microbial communities are located in the large intestine (LI). They are largely controlled by the locus of a resident (autochthonous) bacteria.

Tuberculosis, food poisoning, cholera, pneumonia, sore throat and meningitis: These are just a few of the diseases that are caused by bacteria. Maintaining hygiene both at home and in a clean body is one of the best ways to curb the spread of bacterial infections, but recently, consumers are getting the message that washing with regular soap is not enough.

Antibacterial products have never been so popular. Soaps, household detergents, sponges, mattresses and lip gloss, are now organizing components of bacteria, and scientists are asking themselves where, if any, the daily chemicals of healthy people.

The duration of antibiotic treatment in patients with sepsis, for example, may lead to overuse of antibiotics, which increases the risk of bacterial development. Proctonin (PCT) based antibiotic use reduces exposure to antibiotics in community-acquired pneumonia. If it can also reduce the exposure to antibiotics in severe oxygen is not known.

When a bacterial population is placed under pressure - such as an antibacterial chemical - a small sub-population armed with special defenses can develop. These dynasties survive and replicate as their weaker relatives. "What does not kill you makes you strong" is the conventional rule here, as antibacterial chemicals opt for bacteria that tolerate their presence.

This article refers to current studies on the short-term and long-term effects of the use of antibiotics on human microbiomes, from early life to adulthood, and its impact on diseases such as malnutrition, obesity, diabetes and clostridium inflammation [594].

The production of healthy agricultural products may also suffer from disadvantages from microbiome alterations. Antimicrobial agents are widely used in animal farms to prevent and treat disease in animals and promote growth. Antimicrobial agents may alter the bacterial community and improve animal fecal resistance.

Doctors usually prescribe antibiotics to treat infections. The choice of antibiotics is well specified in clinical guidelines for targeting specific pathogens, Gram (+) bacteria or Gram (-) [595]. However, only little is known to us about the effects of antibiotics on the whole composition and load of gut microbiota immediately after

treatment.

The gut microbiota contains many trillions of bacteria belonging to hundreds, thousands of species, and is essential for optimal preservation of physiological processes. The microbiota protects against other infections and pathologies by directly inhibiting invasive bacteria or by scheduling appropriate immune responses; In contrast, metabolites produced by some intestinal tract can promote a wide range of diseases such as atherosclerosis or cancer.

Antibiotics alter the microbiota blendings, resulting in an increased risk of disease, secondary infections, allergy and obesity. In addition, they promote the spread of patho- resistant gens to drugs, making the search for alternative clinical approaches mandatory. Innovative strategies are developed to replace or complement antibiotic treatments, in an attempt to divert the pathogens without disrupting micro bites and / or recreating the Commons communities along with the shield and under-seduction.

Introduction

The Human Digestive System (GI) includes hundreds of microbial species (microbiota) and thousands of species, and is one of the most densely inhabited ecosystems on Earth [596]. The claim that bacterial cells in the intestines exceed the number of human cells in the body by a ratio of 10:1 was recently modified and suggested to be about 1:1 ratio [597]. However, the actual ratio and number of cells are not as important as the functional capacity of the gut microbiota, which has many positive benefits to host physiology health; Including improved energy harvesting, vitamin synthesis, cell and immune cell modification of vaccine and development, and protection against infection [598]. The breakdown of the average "normal" colon composition has important implications for human health and disease, after being linked to conditions such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), cancer and obesity [599]. In many cases, additional evidence is needed to fully link the microbiota and the disease, and it is not known whether a different microbiota is a cause or a consequence of the disease [600]. In the case of Clostridium-difficile infection (CDI), most cases are undoubtedly related to alteration of the colon composition, usually after administration of antibiotics to the host [601]. Here, we discuss how advances in microbial research revealed new opportunities for control of C. difficile [602].

In the past two decades, the microbiota has been shown in the intestines as a fundamental factor in host physiology and pathology. Trillions of bacteria inhabit the digestive system (GI) of complex metazoans (any multicellular animal), including humans, greatly expanding the host genetic repertoire. It translates the possibility of the host to perform functions that are encoded by its genome: Camps protect against pathogen invasion, extract extra energy from the food, synthesize key molecules for tissue development in a manner that is highly specialized in their position relative to the digestive tract. Although the physiology of all organs is affected by microbiota, the mucous mucosa and its immune components are most affected by this symbiosis. We are reviewing for the first time recent findings that clarify the effect of microbiota on the immune system. Second, we discuss the involvement of intestinal interactions in the pathogenesis of the disease. Thirdly, we examine the role of antibiotics in disrupting or driving these processes. Finally, we discuss the mechanisms of antibiotic development and resistance, as well as the proposed approaches to overcome the drawbacks of antibiotic therapy.

Overuse of antibiotics may fuel the dramatic increase in conditions such as obesity, diabetes, inflammatory bowel disease, allergies and asthma, which have multiplied more than many populations (see graph). We need to urgently explore this possibility. Even before we understand the full scope, action must be taken. Bacteria lived on animals - which constitute their microbial - because molecular life evolved about a billion years ago. The hosts derive many benefits from their bacteriological guests 2: the Bacteroides species living in our colon synthesize required vitamin K; The intestinal bacteria help us to resist invading organisms.

There is other evidence that antibiotics cause a change in the microbial composition that can lead to long-term physiological changes. For example, as farmers have discovered, ongoing, therapeutic under-dosages of many different antibacterial substances cause animals to gain weight with less food. And the longer the antibiotic starts, the deeper the effect. In my laboratory, we have preliminary evidence in a mouse model that changes in body fat and tissue composition are associated with low-dose antibiotic therapy, which imitates farm use and high-dose therapy similar to those used to treat childhood infections. The changes in our microbium may even fuel the transfer of deadly organisms such as staphylococcus aureus, methyciline and clostridium difficile [603]. This is not a surprise, because one of the important roles of an intact microbial ecosystem is to resist intrusions by pathogenic organisms.

Antibiotic-related diarrhea due to Clostridium difficile (CDAD) is considered to reflect colonization of a defective microbial community by the pathogen. Scientists have created a profile of fecal microbiota of patients with CDAD (primary and recurrent episodes) by independent phylogenetic analysis in the culture of gene sequences in 16R rNA gene encoding. Compared to those in the control group, and patients with an initial episode, fecal communities in patients with recurrent CDAD were very variable in the bacterial composition and characterized by significantly reduced diversity. The preservation and restoration of microbial diversity may represent new strategies for the prevention and treatment of recurrent CDAD, which often suffers from existing therapies. Scientists have presented molecular-based ecological evidence for the role that reduced the diversity of bacteria in the case of CDAD. This finding may lead to new treatments and prevention against the newly contagious disease.

Recent discoveries of unexpected variations in the microbial composition of healthy individuals high- light the importance of identifying the processes that could possibly give rise to such variation. Ecological theory seeks to explain and predict observable phenomena, such as temporal and spatial patterns of diversity [604].

Ecological theory seeks to explain and predict observable phenomena, such as temporal and spatial patterns of diversity. The concept put forth in the late nineteenth and early twentieth centuries that 'everything is everywhere, but the environment selects' had a powerful impact on thinking about microbial community assembly, but a more recent appreciation of other ecological processes (such as diversification and dispersal limitation) suggests that

this conceptualization was overly simplistic. Here, we explore how community assembly theory could be used to understand the human-associated microbiota and its role in health and disease. We focus on three scenarios relevant to the assembly of the human microbiome: assembly in previously unoccupied habitats (e.g., postnatal development), reassembly following disturbance (e.g., following antibiotic treatment), and assembly in the context of invasion (e.g., by a pathogen).

The higher the importance of identifying processes that can cause such a change [605].The ecological theory seeks to explain and predict observable phenomena, such as temporal and spatial patterns of diversity.The concept presented at the end of the nineteenth century and the beginning of the twentieth century, "Everything is everywhere, but the environment chooses" has had a strong influence on thinking about microbiological community structure, but a newer assessment of other ecological processes (eg diversity and scattering) indicates that this concept Was too simplistic [606]. Here, we explore how community theory can be used to understand microbiota associated with humans and its role in health and disease. We focus on three scenarios relevant to the human microbial assembly: assembly in previously unavailable habitats (eg postnatal development), re-assembly due to interference (such as antibiotic treatment) and its composition in the context of invasion (eg,).

Adaptive Management of the Human Body

The transition from the clinical practice of the body as a battlefield to the human response as a habitat will necessitate rethinking how man manages the human body. In the management of plant and animal communities, access to the system level known as "adaptive management" has become popular. This approach is a structured and iterative process of decision making that uses system monitoring to update management decisions on an ongoing basis [607]. It has been successfully used to manage biodiversity in a variety of habitats, including communities in highly disturbed environments affected by overfishing by climate change. For the human body, we imagine that this approach will involve assessing microbiology during health, to create a healthy foundation, with more intensive monitoring during disease and treatment. This will require the development of new, accurate, and fast diagnostic tools to inform treatment decisions. Such diagnoses are not yet possible, but given the recent developments in our ability to review the human microbiology, this possibility is not far in the future, especially if we are able to identify certain microbiome components that contribute disproportionately to human health. An adaptive management approach to clinical medicine provides a wonderful example of personalized medicine, with treatments adapted to individuals based on diagnostic changes in the individual microbiome, and continually coordinated through constant monitoring. This intensive approach, guided by ecological theory, has the potential to revolutionize the treatment of the disease [608]. Interest in rebalancing human gut microbiota to treat disease is growing [609]. Diet, antibiotics, probiotics, prebiotics, and fecal microbiota transplants are treatments with reported potential [610]. For ASD-Autistic Spectrum Disorder, however, only temporary symptom improvements have been reported from vancomycin treatment [611], and probiotics have had mixed clinical results with minimal microbiota analysis or long-term follow-up [612]. Contrasting to probiotics which contain a few bacterial species from milk cultures, fecal microbiota transplant (FMT) contains approximately a thousand-bacterial species native to the gut and has helped treat recurrent Clostridium difficile infection [613] and is promising for the treatment of chronic inflammatory diseases such as inflammatory bowel disease [614] and insulin sensitivity [615]. Therefore, ASD's GI and behavioral symptoms may derive, at least in part, from gut microbiota dysbiosis and FMT may effectively rebalance the gut microbiota and alleviate some GI and ASD symptoms.

Advances in the Microbiome: Applications to Clostridium difficile Infection

Clostridium difficile is a major cause of morbidity and mortality worldwide, causing over 400,000 infections and approximately 29,000 deaths in the United States alone every year.

C. difficile is the usual cause of nosocomial diarrhea in the developed world, and in recent years the appearance of hyper red (mainly ribotypes 027 and 078, sometimes characterized by increased production of toxins), epidemic strains and increased number of infections acquired by the community have caused further worry. Antibiotic therapy with metronidazole, vancomycin or fidaxomicin is an initial treatment for C. difficile infection (CDI). However, CDI is unique because the use of antibiotics is also the leading risk factor for the acquisition of CDI or CDI due to repeated eradication of the "normal" gut microbiota. Therefore, there is an urgent need for alternative treatment, not an antibiotic treatment or prevent CDI. Here, we are reviewing a number of such potential treatments which have emerged from advances in microbiome research.

Concluding Remarks

The application of Phage antibacterial therapy is based on a targeted genetic mechanism of bacterial eradication. I many effective treatments in cases of a targeted resistant bacterial strand, like foot abscesses our burns, where mainly one resistant bacteria culture caused the infection, success was achieved based on the unique selectivity of the phages toward the specific bacterium. However, in cases of multiple bacteria-based infections, combined treatment was found as the most effective treatment, Phage, and broad-band antimicrobials agents.

Broad-spectrum antibiotics do allow for the treatment of undiagnosed causative agents with some certainty of success. Conversely, even phages with the broadest bacterial spectrums still do not come close to those of broad-spectrum antimicrobials. However, phage narrow host ranges cannot be assumed to exist in nature. It is evident that the clinical application of bacteriophage-based therapy of resistant bacteria-based infections is only in its infancy.

More efforts should be allocated to do the combined [616] phage-based anti-infective treatments a real-life saver medical practice.

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to the membrane protein, an obstacle that does not exist with the N-Me variant. Placing the agent in such a position in the POPE (1-palmitoyl-2-oleoyl-phosphaethanolamine) layer present in the inner part of the outer membrane to still allow "snorkeling" and to corrupt the outer membrane efficiently.

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