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Medicinal Natural Products: A Disease-Focused Approach

Chapter 3

Antimicrobial Natural Products

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ABSTRACT

Although the first antibiotic, penicillin, was discovered in 1928 from a microbial natural source (a mould, *Penicillium notatum*), there is earlier evidence of using natural materials including moulds and herbs for the treatment of infections. Following the serendipitous discovery of penicillin by Alexander Fleming, there have been hundreds of antibiotics (natural, semisynthetic and synthetic) discovered for clinical uses. However, the pathogenic organisms have developed resistances to existing antibiotics though various mechanisms. Such antibiotic resistance or antimicrobial resistance (AMR) is a critical problem of today's healthcare system urging the development of new antibiotics. This chapter has primarily focused into antimicrobial compounds developed through natural routes that are currently available as antibiotics for clinical uses and/or are at various developmental stages within the drug development pipeline for potential treatment of minor and life threatening infections. The chapter also provides an overview on the catastrophic problem of antimicrobial resistance, its causes, how it spreads as well as modes of developing antimicrobial resistance (AMR).

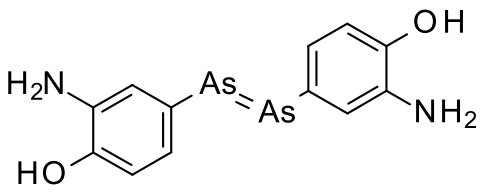
Keywords: Antibiotics; antimicrobial agents: antimicrobial resistance; AMR; natural products; penicillin; antimicrobial peptides; AMPs

1. Introduction

Infectious diseases are one of the major problems in today's healthcare system. Antibiotics have been used since the second world war for the treatment of various types of infections. The term antibiotic is originated from 'antibiosis' that simply describes the interaction of two or more organisms having at least one being detrimental to other(s). Therefore, antibiosis is the process, where one organism in the presence of other organism(s) is capable of producing harmful effects to the later organism(s). 'Antibiotics' literally describes a class of chemical compounds that are responsible for the treatment of infections by inhibiting the growth of bacteria or killing bacteria with minimum or no harm to the host. However, a more broad term 'antimicrobial agents' is used to cover the treatment of infections caused by various organisms including bacteria, fungi, protozoa and virus. Moreover, another term 'anti-infective agent' has also been adopted worldwide in the research of antimicrobial drug discovery.

Even though the fortuitous discovery of penicillin in 1928 started the golden era of antibiotics, the empirical knowledge of treating wounds with the indigenous herbs and/or alternative approaches dates back to the ancient times. Without having any scientific knowledge, wounds were treated with spider's webs, cheese mould or mouldy bread ingestion,[1] mouldy soybean curd and honey.[2] The ancient Egyptians used mouldy bread for the treatment of infected wounds, whilst the ancient Chinese used to apply mouldy soybean curd to heal boils and managed foot infections by wearing sandals furried with mould.[2] In the middle ages, honey was used for the treatment of post-arrow wounds.[3] During this prehistorical era of antibiotics, such treatments were given without any knowledge of bacteria or other organisms that caused the infections.

The search for an effective agent to win in the battle against the bacteria or other organisms causing infections started in late nineteenth century. During an experiment with *Anthrax bacilli* in 1877, Pasteur and Joubert noticed that *Anthrax bacilli* were killed, while contaminated with other bacteria. Another experiment in 1901 revealed that a liquid culture of *Pseudomonas aeruginosa* injected to infected rabbits with anthrax recovered them from anthrax. Such experimental findings led to the conclusion that the metabolite(s) produced by one organism revealed their capabilities to inhibit the growth of other organisms, which supported the concept of antibiosis.[2] Later in 1910, a Nobel laureate Paul Ehrlich discovered an arsenic containing compound known as salvarsan or arsphenamine (**1**) that became the choice of medicine for the treatment of infections including syphilis and trypanosomiasis[3] until it was replaced by first antibiotic penicillin in 1945.



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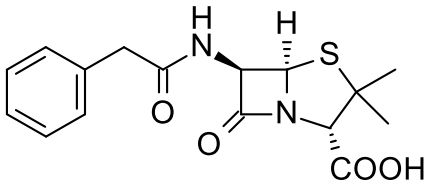
Alexander Fleming, a Scottish Physician and Microbiologist, serendipitously discovered penicillin G (**2**) in 1928. He was working on *Staphylococci* and stacked some petri-dishes of *Staphylococci* on a bench in a corner of his laboratory just before he went for holidays in August in 1928. Upon his return from holidays, he noticed that a fungus had contaminated a culture plate of *Staphylococcus* bacterium he had accidentally left uncovered. He also noticed that the fungus had inhibited the growth of *Staphylococcus* bacterium. Fleming grew the mould in a pure culture and noticed that it produced a metabolite that responded dramatically to treat a number of bacterial infections. He identified the mould as *Penicillium notatum* and named the metabolite as 'penicillin', which became the choice of drug to fight bacterial infections during that period. Alexander Fleming shared the 1945 Nobel Prize in Medicine with Howard Florey and Ernst Chain for their contribution in mass production of the first mass-produced antibiotic. Considering the number of lives saved during the end of World War II, penicillin was nicknamed 'the wonder drug'.[1-3]



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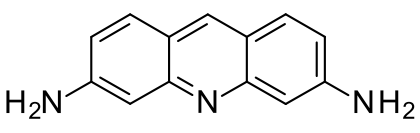
During the World War II, two more drugs proflavine (**3**) and prontosil (**4**) were introduced. Proflavine introduced in 1934 was effective in treating infections with deep surface wounds, however it was highly toxic. The discovery of prontosil, a sulphur-containing prodrug, in 1935 was a real breakthrough for the treatment of systematic infections until the availability of penicillin in early 1940s.[3]



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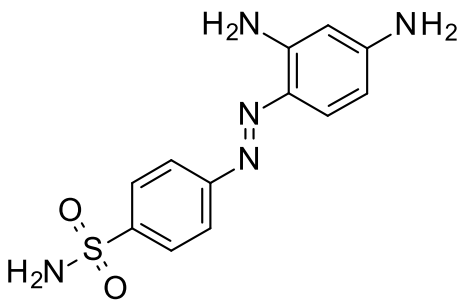

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2. Antimicrobial resistance

Since the discovery of penicillin, there have been hundreds of antibiotics discovered that are in clinical use to treat infections. Considering the availability of hundreds of antibiotics for clinical use, one may raise question why we need new antibiotics. The answer is simple; the pathological organisms (bacteria, fungi etc) have developed resistance to various antibiotics that have been designed to kill them (bugs) through a number of mechanisms (Section 2.2). For example, in 1962, *Staphylococcus aureus* developed resistance against methicillin, which was discovered in 1960. Similarly, because of developed resistance, penicillin became ineffective against *Streptococcus pneumoniae*, and vancomycin against *Enterococcus faecium*,16 years after its discovery (Table 1).[4]

Antibiotic resistance (ABR) or more widely used term antimicrobial resistance (AMR) has become an increasing threat to today's healthcare system because of the unavailability of new and safe antibiotics to respond to the demand of antibiotics to treat increased number of life threatening infections. AMR infections currently counts more than 67,000 infections in Europe with approximately 33,000 death[5] costing around €1.1 billion, whilst there are more than 2.8 millions infections and more than 35,000 AMR related death certificates currently issued in the United State.[6] Methicillin-resistant *Staphylococcus aureus* (MRSA) is a big concern as this is a major cause of both healthcare- and community-associated infections around the world. It has been estimated there are more than 150,000 patients due to MRSA infections in the Europe, which costs approximately €380 million for EU healthcare systems.[7] Pan-European surveillance data on bloodstream infections showed that more than 10% *Staphylococcus aureus* infections in 15 European countries are due to MRSA with some of these countries seeing such resistance closer to 50%.[8] If no action is taken on tme, it has been estimated that AMR will be the leading healthcare problem counting in excess of 10 million deaths per year by 2050 costing the world an extra $100 trillion.[9]

Table 1: Antibiotics timeline with dates of discovery and development of resistance[4]

Antibiotics	Year discovered	Resistant organisms	Year developed
Penicillin	1941	Penicillin-resistant *Staphylococcus aureus* Penicillin-resistant *Streptococcus pneumoniae* Penicillinase-producing *Neisseria gonorrhoeae*	1942 1967 1976
Tetracycline	1950	Tetracycline resistant *Shigella*	1959
Amphotericin B	1959	Amphotericin B-resistant *Candida auris*	2016
Methicillin	1960	Methicillin-resistant *Staphylococcus aureus*	1962
Vancomicin	1972	Plasmid-mediated vancomycin-resistant *Enterococcus faecium* Vancomycin-resistant *Staphylococcus aureus*	1988 2002
Azithromycin	1980	Azithromycin-resistant *Neisseria gonorrhoeae*	2011
Ciprofloxacin	1987	Ciprofloxacin-resistant *Neisseria gonorrhoeae*	2007
Fluconazole	1990	Fluconazole-resistant *Candida*	1988
Daptomycin	2003	Daptomycin-resistant methicillin-resistant *Staphylococcus aureus*	2004
Ceftazidime-avibactam	2015	Ceftazidime-avibactam-resistant KPC-producing *Klebsiella pneumoniae*	2015

2.1 Antimicrobial resistance: its causes and ways it spreads

Antimicrobial resistance has become a catastrophic problem for public health in the recent years. Widespread uses of antibiotics in medicine, veterinary, agriculture and poultry have contributed a lot toward the development of bacterial resistance. The various causes beyond the development of resistance to the antibiotics are briefly summarized below.

2.1.1 Genetic modification of organisms: Antimicrobial resistance can happen naturally over time, usually through genetic changes in the bugs. Within our body, there are lots germs-some considered as good bacteria (protect the body from infections), other as bad bacteria (cause illness through infections) and only few as drug resistant bacteria. When antibiotics are taken, they usually kill both bad and good bacteria but cannot do any harm to the drug resistant bacteria which in turn are allowed to take over and grow. Some drug resistant bacteria give their resistance gene to other bacteria making the latter group of bugs resistant and causing the problem worse. Thus, antibacterial resistance occurs naturally through genetic modification.[10]

2.1.2 Over prescription of antibiotics: Antibiotics have been misused and/or overused in humans and animals, which has accelerated dramatically the problem of antimicrobial

resistance. Sometimes antibiotics are given for viral infections like cold and flu which do not require antibiotics at all. Again, antibiotics are unreasonably given to animals for growth promotion and/or prevention of diseases in healthy animals. Antibiotics are also used unreasonably in agriculture and for the preservation of poultry products. Such misuses and/or overuses of antibiotics cause AMR.

2.1.3 Gratuitous prescription of broad spectrum antibiotics: Sometimes broad spectrum antibiotics are prescribed for the conditions, which could be treated with a narrow spectrum antibiotics. Such unjustified prescriptions also contributes towards the development of antimicrobial resistance.

2.1.4 Over-the-counter access to antibiotics and self-medication: Unrestricted access to antibiotics and self-medication have made AMR situation even worse in developing countries. Patients in some developing countries can buy antibiotics without any prescription which enable them to get hold of antibiotics whenever they wish to do so. Thus, they take antibiotics without consulting their physicians for conditions, where they should not require antibiotics at all. Sometimes they start taking antibiotics but stop before completing the course. Such irrational uses of antibiotics are an important route of developing AMR in some developing countries. However, AMR awareness activities have recently motivated the policy makers in developing countries to think carefully to take initiative to stop antibiotics without prescriptions.

2.1.5 Antibiotics trafficking: Because of easily availability, some people buy antibiotics when they travel to developing countries and carry antibiotics with them when they come back. Upon return they might consider taking antibiotics for some conditions even through the physicians have not prescribed antibiotics. Such ignorance hastens the problems of antimicrobial resistance.

2.1.6 Antimicrobial manufacturing contaminations: During the manufacturing antibiotics (both raw materials and finished products) in the Pharmaceutical Industries, antibiotics manufacturing wastes and effluents contaminate the environments (air, soil and water) and become a major concern for antimicrobial resistance as well.

Now the next concern regarding AMR is how it spreads. There are two ways of spreading AMR- (i) human to human and (ii) animals (poultry) to human. If a person takes antibiotics for the treatment of infection, but unfortunately develops resistant bugs, then the person may stay at home or gets care in hospital, care home or other inpatient facilities. If s/he stays at home, then AMR may spreads to family members and/ or friends and ultimately to the

community. At hospital or inpatient facilities, the infected person spreads AMR to doctors, nurses and other health professionals and thereby to the community.

When animals (common poultries) are given antibiotics but developed resistant bacteria in their guts, they spread these resistant gene to people when the meats from animals with drug-resistant bacteria are not handled properly and/ or cooked properly. Sometimes, fertilizers or water containing animal faeces and drug-resistant bacteria are used to grow vegetables and crops and thereby, such drug-resistant bacteria can be transferred to vegetables and crops. Ultimately, such drug-resistant bacteria spreads to humans when these vegetables and crops are consumed.

2.2 Mechanisms of antimicrobial resistance

Although several classes of antibiotics are in clinical uses and act in different modes of action, however the organisms have developed resistance to existing antibiotics using one or more of the following mechanisms.

2.2.1 Enzymatic inactivation of the antibiotics: This is the most common mechanism of developing resistance to several antibiotics such as penicillins, cephalosporins, chloramphenicol and aminoglycosides. This is simply happens when bacteria (both Gram-positive and Gram-negative) are capable of producing enzymes that inactivate or destroy the antibiotics. For example, β-lactamases enzymes produced by *Staphylococci* and also, sometime Gram-negative bacteria, cleave the β-lactam ring of penicillins and cephalosporins.[11,12] Some of these β-lactamases encoded by transposons may transfer resistance genes to other antibiotics as well. Another enzyme, chloramphenicol acetyltransferase, produced by both Gram-positive and Gram-negative bacteria inactivate chloramphenicol.[11] Similarly, kinases and other enzymes produced by both Gram-positive and Gram-negative bacteria can inactivate aminoglycosides by enzymatic reactions like phosphorylation, adenylation or acetylation.[11,13]

2.2.2 Alteration of antibiotic target sites: A large number of target sites alterations mechanisms are also found in clinical isolates. Chromosomal mutations can alter 30S subunit of ribosome which is the binding site protein for aminoglycoside and thereby, inactivate the antibiotic. Similarly, erythromycin may be inactivated through a plasmid-led changes on the 50S subunit of ribosome. Rifampicin resistance was reported to be related to the DNA-dependent RNA polymerase alteration.[11,13] Besides the enzymatic cleavages, resistance to β-lactam antibiotics is also conferred by target modification through a mutated chromosomal

gene as seen in MRSA strains where its exogenous penicillin binding protein (PBP; its transpeptidase domain) is insensitive to the action of several different β-lactams. [11,13]

2.2.3 Decreased intracellular accumulation and/or efflux of antibiotics: This is considered as second common modes of developing antibiotic resistance in clinical strains. Decreased permeability is prominent in Gram-negative bacteria because of the presence of the additional outer membrane favouring a permeability barrier and offering an essential mechanism for protection against hydrophilic antibiotics like vancomycin.[14] Plasmid-determined inhibition of the porin genes and/or changes in their expression have been evident to further impact the susceptibility of Gram-negative bacteria to hydrophilic antibiotics.[15] Both Gram-positive and Gram-negative bacteria have also been reported to exhibit different types of active efflux pumps belonging to one of the five families: ABC, MFS, RND (Resistance-Nodulation-Division), MATE (Multidrug and Toxin Extrusion), and SMR (Small Multidrug Resistance).[16] The emergence of resistance to fluoroquinolone in *Streptococcus pneumoniae* has been evident by active efflux pumps.[17]

2.2.4 Modification of metabolic pathways or bypassing antibiotic inhibiting reactions: Plasmid mediated sulphonamide resistance in many bacteria appears due to the production of dihydropteroate synthetase with low affinity for antibiotic but no alteration in the affinity for PABA.[11] So sulphonamide resistance bacteria do not use PABA to synthesize nucleic acid and folic acid.[18]

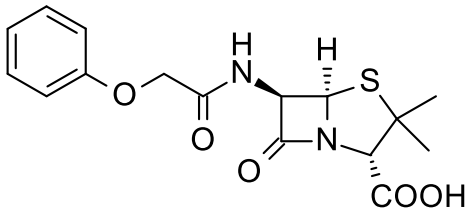
3. Antimicrobial natural products

Since the dawn of human civilisation, human beings have depended on natural resources, mostly plants and moulds, for the treatment of various types of diseases including infections. Although the majority of the today's antibiotics are derived from microbial sources or their semisynthetic analogues, scientists around the globe have continued to carry out systematic research on microbes as well as on medicinal plants, marine organisms and animals for the discovery of new antibiotics. This section highlight key antimicrobial compounds isolated from natural sources covering microbes, medicinal plants, marine organisms and animals.

3.1 Antimicrobial natural products from microorganisms

Penicillin G (benzylpenicillin, **2**) and its semisynthetic analogue penicillin V (phenoxymethylpenicillin, **5**) belong to the class of β-lactam antibiotics that act by inhibiting the synthesis of the bacterial cell wall peptidoglycan. These are active against a wide range

of organisms and considered as drugs of first choice for various infections. However, the poor absorption in the gastrointestinal tract and susceptibility to the enzyme β-lactamase are their main shortcomings.



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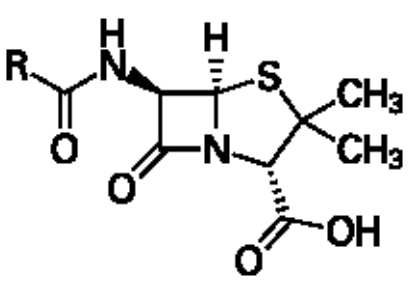
A number of further semi-synthetic penicillins such as methicillin, flucloxacillin, ampicillin and amoxycillin were developed by simple modification of side chains attached to the penicillin nucleus (R in Figure **1**). Among these, methicillin and flucloxacillin are β-lactamase resistant penicillins, while ampicillin and amoxycillin provide broad spectrum activity.[19]

Cephalosporins also belonging to β-lactam antibiotics were originated from the fungus *Cephalosporium*. Discovered in 1945 by an Italian pharmacologist Giuseppe Brotzu, cephalosporins are broad spectrum antibiotics used for the treatment of a number of infections including septicaemia, pneumonia, meningitis, biliary-tract infections, peritonitis, and urinary-tract infections. Cephamycins, structurally similar to cephalosporins, are also classified as β-lactam antibiotics having similar mechanism of action as penicillins which were isolated from the microorganism *Streptomyces*, the largest genus of Actinobacteria. Various semisynthetic cephalosporins were developed by modifying the two side chains (R_1 and R_2 in Figure **1**) of cephalosporin nucleus. [19,20]



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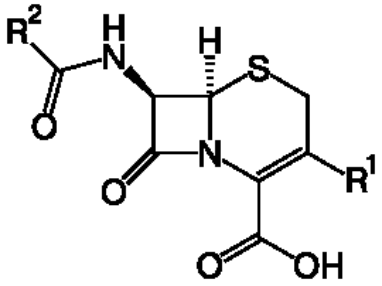

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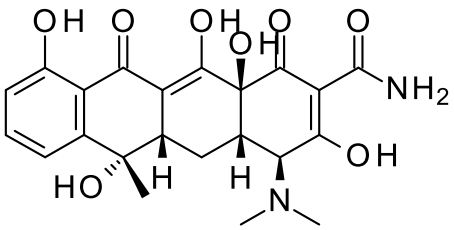
Figure 1: General structures of penicillins (**6**) and cephalosporins (**7**)

Vancomycin, a glycopeptide antibiotic with seven peptides present in the molecule, was discovered a Pharmaceutical company Eli Lilly in 1952 from a soil bacterium, *Streptomyces orientalis*.[21] It works by inhibiting the bacterial cell wall synthesis in the last stage through binding to the terminal D-alanyl-D-alanine of peptidoglycan precursors. This is used for the treatment of MRSA and some other bacteria resistant to β-lactam antibiotics. Its clinical uses also include the treatment of infections related to *Pseudomonas colitis*.[21]

Tetracyclines (**8**) discovered in the 1940s are a class of broad-spectrum antibiotics, which following entry into the organisms act by interfering the bacterial protein synthesis. They consist of four six-membered rings (designated as A, B, C and D) fused linearly to form tetracycline (four cyclic) nucleus to which various functional groups and/ substituents are attached. As they contain a number of keto groups in the molecules they are also considered a class of compounds known as polyketides. Members belonging to this antibiotic class include tetracycline, oxytetracycline, chlortetracycline, demeclocycline, minocycline, doxycycline, methacycline and lymecycline. Among these, chlortetracycline and oxytetracycline are the first two members of natural tetracyclines both discovered in the 1940s from the filamentous bacteria *Streptomyces aureofaciens* and *S. rimosus*, respectively. Other naturally occurring antibiotics in the class such as tetracycline were isolated from *S. aureofaciens, S. rimosus*, and *S. viridofaciens* and demethylchlortetracycline from *S. aureofaciens*.[22]



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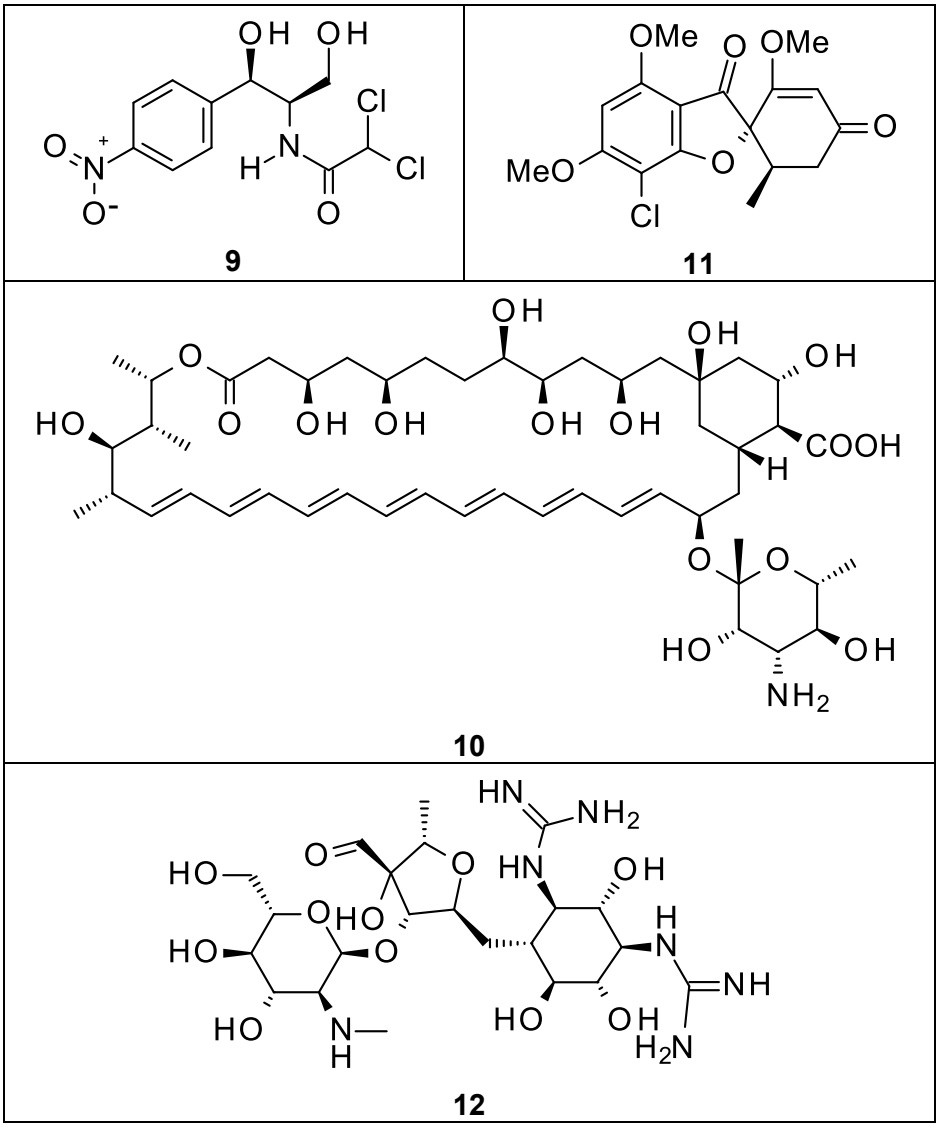
Semi-synthetic tetracyclines such as doxycycline, minocycline, methacycline and lymecycline are produced from natural tetracyclines by simple modifications on the functional groups and/or substituents. Tetracyclines are used for the treatment of a wide range of microorganisms including both Gram-positive and Gram-negative bacteria, *Chlamydia*, *Rickettsia*, *Mycoplasma* and protozoa.[22] Because of their capabilities of reacting with chelating ions such as calcium, magnesium, aluminium and iron forming non-absorbable complexes, their absorption is reduced significantly in the presence of milk, iron preparations and antacids. Accordingly, patients are advised not to take oral tetracyclines with milk, antacids and iron preparations to avoid the consequence of forming non-absorbable complexes.

Chloramphenicol (**9**), an amphenicol, was isolated from a soil-dwelling bacterium, *Streptomyces venezuelae* in 1947 and its chemical structure was confirmed in 1949.[23] Following its discovery, it was manufactured in large scale through synthetic route and is considered as the first synthetic antibiotic as well. It acts by interfering the protein synthesis of bacteria by binding to the 50S ribosomes. It has a wide range of antimicrobial activity including Gram-positive and Gram-negative bacteria as well as rickettsia. Chloramphenicol ointment is widely used for the treatment of eye infections.[24] Together with antibiotics such as amphotericin B (**10**), griseofulvin (**11**) and streptomycin (**12**), chloramphenicol (**9**) is in the World Health Organisation's List of Essential Medicines, the safest and most effective medicines needed in a health system.[25] However, its most common side effect is idiosyncratic depression of bone marrow. It also causes grey baby syndrome in young children so it should administered to new-borns with great care by regular monitoring its plasma level.[24]

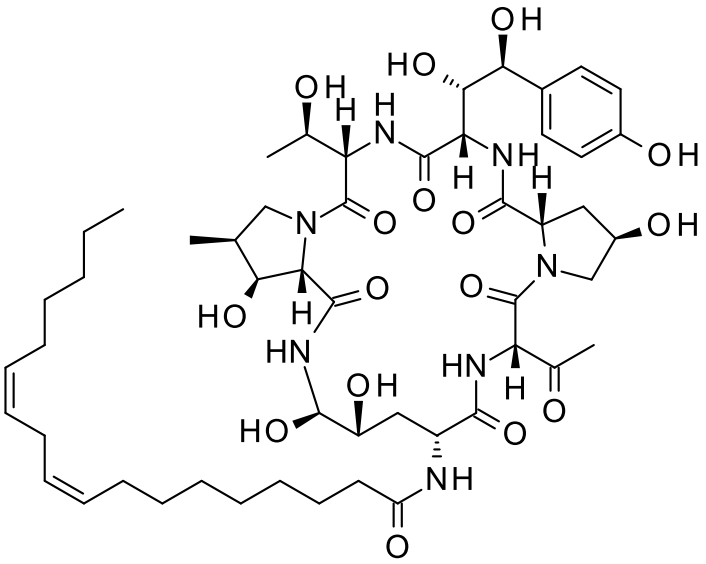


Amphotericin B (**10**) is an antifungal agent derived from the culture of *Streptomyces nodosus* in 1955.[26] This is a polyene macrolide that acts on the fungal cell membrane and binds with its cell membrane component ergosterol forming large pores leding to the gross disturbance in monovalent ion balance with ultimate leakage of intracellular K^+, Na^+, H^+ and Cl^- resulting in fungal cell death. This is a preferred antibiotic for the treatment for disseminated infections caused several fungi and yeast including *Aspergillus* and *Candida*.[27]

Griseofulvin (**11**), discovered in 1939 from a culture of *Penicillium griseofulvum*,[28] is a narrow spectrum antifungal antibiotic that works by binding to fungal microtubules and thus inferring fungal mitosis. It is administered orally for the treatment of dermatophytosis including the fungal infections of skins, scalp and nails when the local/ topical antifungal agents become ineffective.

Streptomycin (**12**) belongs to the aminoglycoside class of antibiotics, and was discovered in 1943 from a culture of soil actinomycete, *Streptomyces griseus*.[29] Kanamycin (also known as kanamycin A) is another aminoglycoside antibiotic which was isolated in 1957 from the soil actinomycete, *Streptomyces kanamyceticus*.[29] Chemically, these aminoglycosides contain aminated carbohydrate rings connected to dibasic cyclitol through glycosidic linkage. Upon uptake to susceptible organisms, these antibiotics bind to the bacterial 30S ribosomal subunit and thereby disrupt the initiation and elongation steps in protein synthesis. As broad spectrum antibiotics, these are used for the treatment of several life threatening infections including tuberculosis, sepsis, endocarditis, brucellosis, and severe urinary tract infections.[30]

Echinocandin B (**13**) is an antifungal agent which is composed of a ring of six amino acids connected to a long-chain lipophilic side chain. It was discovered in 1974 from a culture of *Aspergillus nidulans*.[28,31] It acts by inhibiting the synthesis of 1,3-β-glucan, an essential component of fungal cell wall structure. It is fungicidal against some yeasts, mostly *Candida* species. Bacitracin is another similar polypeptide antibiotic composed of 11 amino acids (seven as part of ring and four in the side chain) was discovered in 1945 from a culture of the licheniformis group of Gram-positive bacterium *Bacillus subtilis*.[32] It is a narrow-spectrum antibiotic used for the treatment of infections caused by Gram-positive bacteria, especially those that cause skin infections specially those caused by small cuts, scrapes, or burns. It stops the growth of certain bacteria by inhibiting the bacterial cell and peptidoglycan synthesis.[32]



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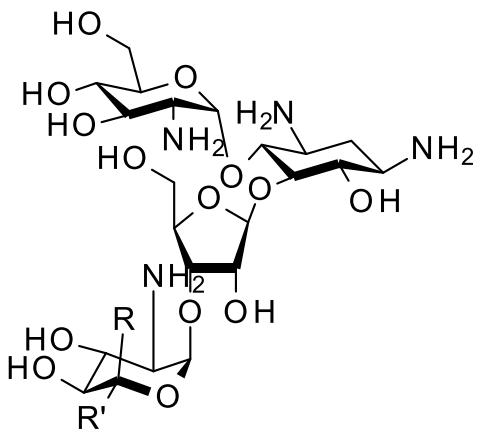
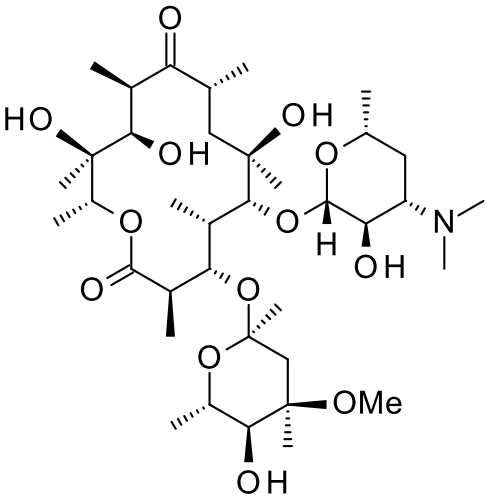
Polymyxins are cationic polypeptides composed of a cyclic heptapeptide plus a tripeptide side chain acylated at the *N* terminus by a fatty acid tail. Polymyxin B was first isolated in 1947 from a culture of a Gram-positive bacterium, *Bacillus polymyxa.*[33] Colistin (also known as polymyxin E) is a polypeptide antibiotic that was originally isolated in 1947 from the soil bacterium *Paenibacillus polymyxa* subsp. *colistinus*. [34] Colistin and polymyxin B are used for the treatment of infections caused by Gram-negative bacteria and act by breaking down the cytoplasmic membrane causing the ultimate death of bacterial cell.[33,34]

Neomycins (e.g., neomycin B, **14** and neomycin C**, 15**) are amino glycoside antibiotics comprising amino sugars connected through glycosidic linkages. The first member of this class was discovered in 1949 by the microbiologists Waksman and Lechevalier from a culture of the bacterium, *Streptomyces fradiae*.[35] This antibiotic is available in a number of topical preparations such as creams, ointments, and eyedrops. It acts by inhibiting the protein synthesis through binding with 30S ribosome causing genetic disruption. It also act by interfering the bacterial enzyme DNA polymerase.[35]

Nystatin (**16**), structurally related to amphotericin B, is an antifungal agent, which was isolated from the culture of actinomycetes, *Streptomyces noursei*, found in the soil of a dairy farm in USA.[36] Like other antifungal agent such as amphotericin B, it acts by binding with fungal cell membrane component, ergosterol, forming large pores in the membrane leading the leakage of K+ and ultimate death of fungal cell. It is given for the treatment of fungal infections caused mostly by Candia including esophageal candidiasis, thrush and vaginal infections. [37]



14 R = CH_2NH_2; R' = H
15 R = H; R' = CH_2NH_2



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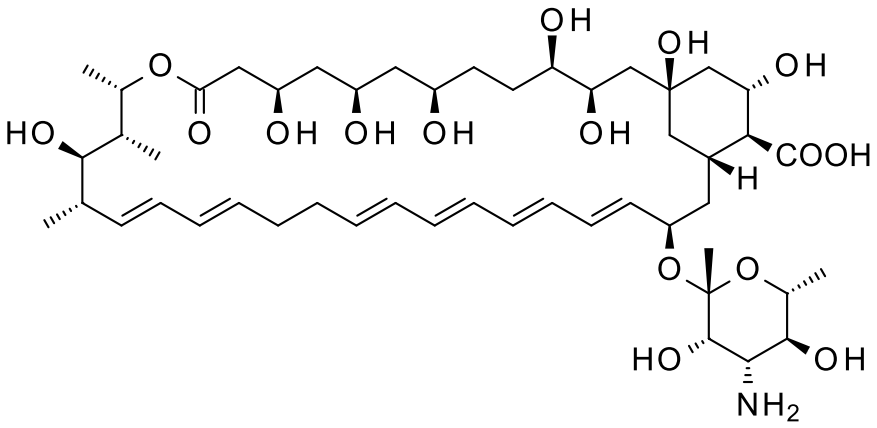

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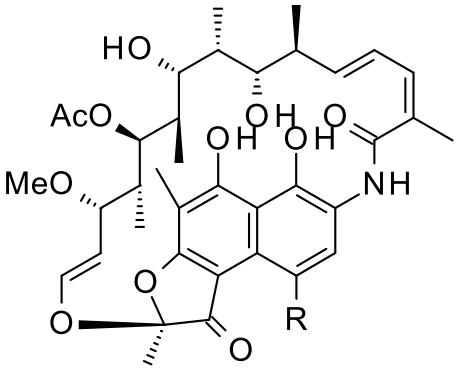
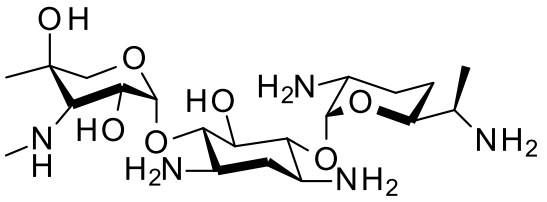


18 R = H
19 R = CH_2COOH



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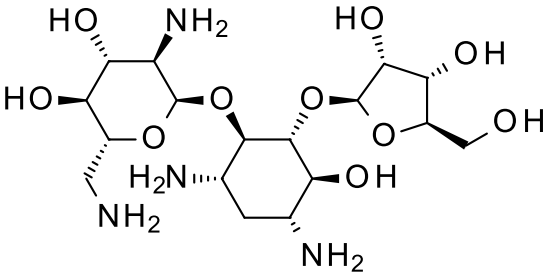


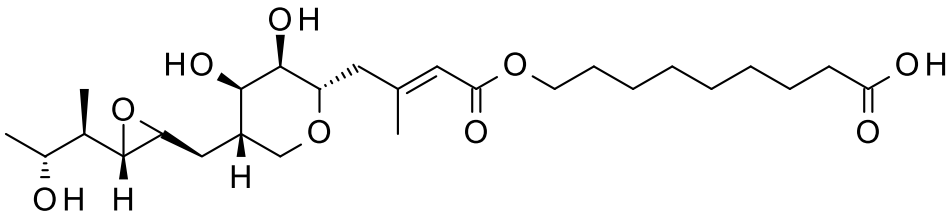
21

Erythromycin (**17**), a macrolide antibiotic, was discovered in 1952 from the culture of bacterium *Saccharopolyspora erythraea*.[38] It offers bacteriostatic activity by inhibiting the protein synthesis through binding with bacterial 50S subunit of rRNA, and is widely used for the treatment of chest infections (pneumonia), skin diseases (acne) and sexually transmitted diseases.[38]

Rifamycins (A-E), a group of structurally related secondary microbial metabolites, were discovered in 1957 from a product of fermentation from the Gram-positive bacterium *Amycolatopsis mediterranei* (also known as *Streptomyces mediterranei)*.[39] Among these metabolites, rifamycin B (**18**) was isolated in pure form but with poor activity, but could easily be oxidized to more active form, rifamycin S, which was further modified to produce clinically relvant rifamycin SV (**19**), the first antibiotic used intravenously for the treatment of tuberculosis. Because of its high affinity to the prokaryotic enzyme, RNA polymeraze, rifamycins act by inhibiting the bacterial DNA-dependent RNA synthesis.[40]

Like neomycins, gentamycin (**20**) and ribostamycin (**21**) are two other popular aminoglycoside antibiotics. Gentamicin (**20**) was identified in 1962 from the fermentation broth of a bacterium, *Micromonspora purpurea*.[41] This bactericidal antibiotic acts by binding the 30S subunit of the bacterial ribosome and thereby, inhibiting bacterial protein synthesis. It is active against a wide range of bacterial infections including urinary tract infections, respiratory tract infections, blood, bone and soft tissue infections.[41] On the other hand, ribostamycin (**21**) was first isolated in Japan in 1970 from the fermentation broth of a soil bacterium, *Streptomyces ribosidificus*.[42] Like other aminoglycosides, it also acts by inhibiting protein synthesis through binding with 30S subunit of bacterial ribosome.[43]

Mupirocin (**22**) was initially isolated in 1971 from the culture of a rod-shaped Gram-negative bacterium, *Pseudomonas fluorescens*.[44] It works by reversibly binding to the isoleucyl t-RNA synthetase in *Staphylococcus aureus* and *Streptococcus* and thereby inhibiting the protein synthesis. This topical antibiotic is used for the treatment of skin infections such as impetigo and folliculitis as well as for MRSA.[44]



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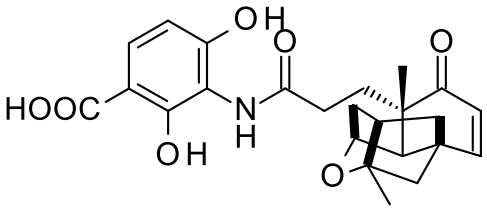
Among the recently introduced antibiotics, daptomycin, a lipopeptide antibiotic, was isolated from the soil bacterium, *Streptomyces roseosporus*.[45] It was approved in 2003 by the FDA for the treatment of complicated skin and soft tissue infections. It acts by disrupting multiple aspects of bacterial cell membrane function. Upon ingestion, it attacks the cell membrane (phosphatidylglycerol) forming holes that leak ions like K^+ and ultimate death of bacterial cell.[45] Platensimycin (**23**) and platencin are relatively recent novel antibiotics which were isolated from a bacterium *Streptomyces platensis* by using an antisense whole-cell differential sensitivity assay, where control organisms were compared to cells expressing fabF antisense RNA.[46] Both compounds a 3-amino-2,4-dihydroxy-benzoic acid and a different unusual diterpene (tetracyclic enone acid in platensimycin, while a tricyclic enone acid in platencin). They are potent and non-toxic natural products with potent activity against Gram-positive pathogens, including antibiotic-resistant strains and *Mycobacterium tuberculosis*.[47] Because of their unique structural features and promising antibacterial activity, they have been a breakthrough in the searches for novel antibiotics. Platensimycin was first isolated by the Merck group.



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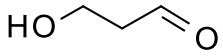
Lactic acid bacteria are good gut bacteria that produce secondary metabolites with potential antimicrobial activity. For example, nisin, which is a polycyclic antibacterial peptide composed of 34 amino acids in the molecule, was produced from the culture of a Gram-positive bacterium *Lactococcus lactis* that has been has been extensively used in the production of buttermilk and cheese. It is used as a food preservative because of its bactericidal activities against Gram-positive as well as spore forming food-borne bacteria including *S. aureus* and *Listeria monocytogenes*.[48] In the research laboratories, it is very useful as a selective agent in microbiological media for the isolation of gram-negative bacteria, yeast, and moulds. Reuterin (3-hydroxypropionaldehyde; **24**),[49] reutericin 6[50] and reutericyclin[51] are produced by *Lactobacillus reuteri* and have a broad spectrum activity against food-borne pathogens and spoilage organisms. Reuterin (**24**), a simple aldehyde, in combination with nisin offer synergistic antimicrobial activity and reduce significantly the growth of Gram-positive *Staphylococcus aureus*, *Lactobacillus monocytogenes*, and Gram-negative *E. coli* and *S. Typhimorium*. Reuterin inhibits the growth of some harmful bacteria (both Gram-positive and Gram-negative) as well as some fungi, yeasts and protozoa. *L. reuteri* secreting

sufficient amounts of reuterin to achieve the desired antimicrobial effects is capable of eliminating gut invaders without harming other gut microbiota.[49]



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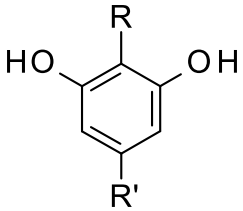
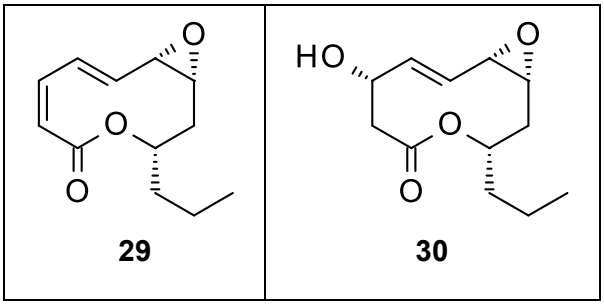
The above examples show the histrory and progression of development of antibiotics from microbial soirces throughout the past several decades, but in the recent past, there are hardly any new antiobiotics reported from microbial sources, which have become commercialy available for the treatment of infections. However, this does not mean the the search for new antibiotics or antimicrobial agents has stopped. In fact, there are several papers published in recent years describing various antimicrobial compounds from microbial sources with diffrent levels and spectrum of activities. A cultural broth of *Pseudomonas* sp. Ki19. was reported to produce four dialkyl resorcinols, 2-butyl-5-propylresorcinol (**25**), 2-hexyl-5-methylresorcinol (**26**), 2-hexyl-5-propylresorcinol (**27**), and 2-hexyl-5-pentylresorcinol (**28**) with antimicrobial activity against *S. aureus* (MIC 10 μg/mL), *Aspergillus fumigatus* (MIC = 50 μg/mL) and *Fusarium culmorum* (MIC = 50 μg/mL).[52]



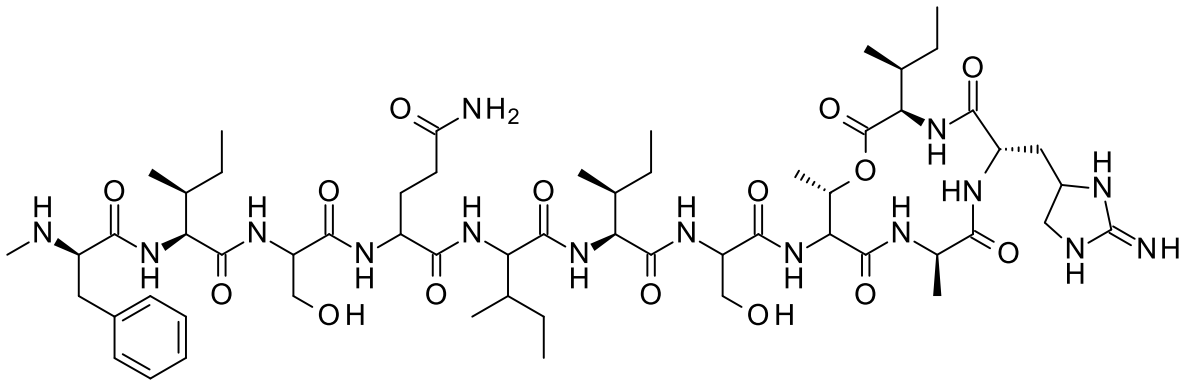
25 R = Butyl; R' = Propyl
26 R = Hexyl; R' = Methyl
27 R = Hexyl; R' = Propyl
28 R = Hexyl; R' = Pentyl

A fungus belonging to the genus *Phoma* produced three acetylenic acids, phomallenic acids A–C, which exhibited potent antibacterial activity against wild-type *S. aureus* with MICs in the range 3.9–7.8 μg/mL, with phomallenic acid C being the most active one, superior to commonly used FabF inhibitors such as cerulenin and thiolactomycin.[53] Ten-membered macrolides, phomolides A (**29**) and B (**30**), were isolated from the cultural broth of *Phomopsis* sp. hzla01-1 which revealed significant antimicrobial activities against *Escherichia coli* CMCC44103, *Candida albicans* AS2.538 and *Saccharomyces erevisiae* ATCC9763 (MICs 5–10 μg/mL).[54]



29 **30**

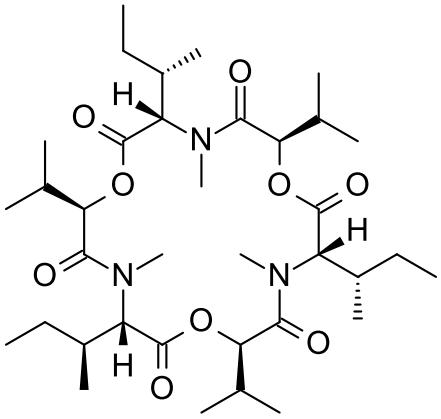
A culture broth of *Lysobacter* sp. produced antimicrobial polypeptides (AMPs), tripropeptins A-E and Z (MICs 0.39–12.5 μg/mL). Among these polypeptides, tripropeptins C and D displayed strong activities against Gram-positive bacteria, including both MRSA and vancomycin-resistant *Enterococcus*.[55] The culture broth of a cyanobacterium belonging to the genus *Hassallia* yielded braod-spectrum antifungal glycosylated lipopeptide hassallidins A and B (MICs of 8-16 μg/mL against 10 species of *Candida*).[56,57] The isolation of an unusual depsipeptide, teixobactin (**31**), from a soil bacterium is the most recent breakthrough in the search for antimicrobial drugs because of its intrinsic antibacterial activity, structural novelty and method of identification involving the culture production in natural soil environment. The hypothesis for thus drug discovery was based on that fact that the uncultured bacteria making up approximately 99% of all species in external environments could be an untapped source of new antibiotics. Accordingly, teixobactin (**31**) has been discovered through a screen of uncultured bacteria using a new device, i-Chip sealed with semi-permeable membranes.[58] It showed potent *in vitro* antimicrobial activity against Gram-positive bacterial strains including *Staphylococcus aureus*, *Bacillus anthracis*, *Enterococci* species and *Clostridium difficile* as well as *Mycobacterium tuberculosis*. It was also found effective *in vivo* against methicillin-resistant *Staphylococcus aureus* and *Streptomyces pneumoniae* in mice model. This compound was demosntrated to act by inhibiting the bacterial cell wall synthesis by binding at both lipid II (precursor of peptidoglycan) and lipid III (precursor of cell wall teichoic acid).[58]



31

The first synthetic analogue of natural teixobactin was synthesized by substituting the L-allo-enduracididine residue with the naturally occurring L-arginine which exhibited the antibacterial activity against Gram-positive bacteria similar to that of teixobactin.[59]

Six-membered cyclic depsipeptides, enniatins [A (**32**), A_1, B, B_1 and B_2], were isolated from the methanolic extract of the endophyte *Fusarium tricinctum* Corda. The methanol extract of *F. tricinctum* displayed mild antibacterial and antileishmanial activities as well as inhibition of the activity of thioredoxin reductase enzyme of *Plasmodium falciparum*.[60]



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A co-cultivated fungus, *Coprinopsis cinereal*, produced a olypeptide antibiotic, copsin, which revealed its bactericidal property against a diversity of Gram-positive bacteria, including human pathogens such as *Enterococcus faecium* and *Listeria monocytogenes*.[61] Similarly, albicidin, a unique polyaromatic oligopeptide, mainly composed of *p*-aminobenzoic acids, was reported from the sugarcane invading bacterium *Xanthomonas albilineans*. This is a potent DNA gyrase inhibitor against a range of both Gram-positive and Gram-negative bacterial strains.[62] Three highly potent novel antibacterial compounds, the myxobacteria-derived cystobactamids 1-3, were isolated from *Cystobacter* sp.[63] These are inhibitors of bacterial DNA gyrase (type II topoisomerase) and revealed activity against *E. coli*, *A. baumannii*, *E. faecalis*, *S. aureus* and *S. pneumonia* with very lows MICs.[63] Despite promising antimicrobial activities of recently repprted compounds, none of then has entered any proper clinival trials or extensive in vivo studies involving various animal models.

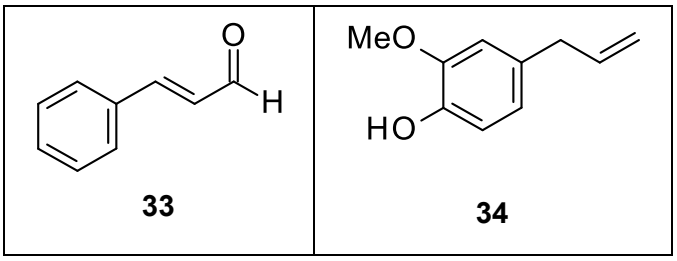
3.2 Plant derived antimicrobial natural products

Plants are main natural remedies which have been used for centuries for the treatment of various human ailments including infections. They are well known for the production of

biologically active compounds and have played key role in modern drug discovery (See *Chapter 1 Medicinal Natural Products – An Introduction* by Nahar and Sarker). A huge number of drugs in clinical uses today are either derived from plants or synthetic analogues of plant derived secondary metabolites. Some examples of such plant derived medicines include anticancer drugs like taxol, vinblastine, vincristine; antimalarial drugs like quinine, quinoline, artemisinin; analgesic drugs like morphine, codeine, eugenol; and cardioactive drugs like digoxin, digitoxin, lactoside C; CNS stimulants like caffeine; laxatives like sennosides and so many other drugs.[64] However, medicinal plants have been underexploited to some extent as a source of antimicrobial lead compounds. Nevertheless, there has been a significant body of ongoing research involving medicinal plants that have traditinally been used for the treatment of various infections in the traditional medicines including thr Ayurvedic system[65] and the Traditional Chinese Medicine (TCM).[66] The antimicrobial property of plants is associated with and a part of their defense mehcanisms against micorbial attacks. Extensive phytochemical and/or bioassay directed syudoes on various medicinal plants have led to the characterization of a wide range of antimicrobial compounds. This section covers key plant-derived compounds with potential antimicrobial activity.

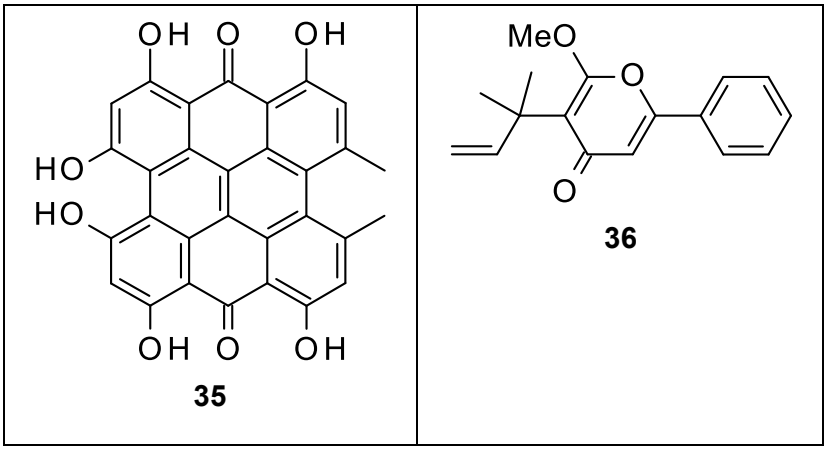
Aromatic medicinal plants, such as cinnamon, clove, cilantro, coriander, fennel, oregano, peppermint, rosemary, thyme etc. are good sources of essential oils, which have been well documented for thier abilities to inhibit the growth of a variety of microorganisms.[67] Such naturally occurring essential oils act as preservatives (inhibit the growth of microorganisms) and flavouring agents and are sometimes incorporated in the food products. Chemically, these essential oils are terpenes, predominately mono- and sesqui-terpenes. For example, the major compounds present in cinnamon, *Cinnamomum zeylandicum*, and clove, *Syzgium aromaticum*, are *trans*-cinnamaldehyde (**33**) and eugenol (**34**), respectively; both are simple monoterpenes. Such essential oils can inhibit the growth of moulds, yeasts, and bacteria. Cinnamon and clove oils added at a concentration of 2% in potato dextrose agar (PDA) displayed complete inhibition of the growth of mycotoxigenic moulds such as *Aspergillus flavus*, *A. parasiticus*, *A. ochraceus*, *Penicillium* sp. M46, *P. roqueforti*, *P. patulum*, and *P. citrinum* for a period of up to 21 days.[68] Both oils have also been documented for their ability to inhibit the growth of many microorganisms including *Lactobacillus* sp., *Bacillus thermoacidurans*, *Salmonella* sp., *Coryne bacterium michiganense, Pseudomonas striafaciens*, *Clostridium botulinum*, *Aspergillus* sp., *Canninghamella* sp., *Fusarium* sp., and *Penicillium* sp.[69] The cinnamon oil was reported to inhibit the growth of *A. flavus*, *A. parasiticus*, *A. ochraceus*, and *Fusarium moniliforme* on PDA at very low concentration (500 ppm).[70] Eugenol, the main constituent of clove oil, has been used widely in perfumeries as flavouring agents, and also as an analgesic, local anaesthetic, anti-inflammatory, and antibacterial

agent.[71] It is used as an important constituent in the formulation of a paste or mixture as dental cement, filler, and restorative material local antiseptic and anaesthetic in dentistry. Clove has also been reported to have strong antibacterial activity against *S. aureus*, *Escherichia coli* and *Listeria monocytogenes* with MICs in the range of 0.3-2.5 μg/mL.[72]



33 **34**

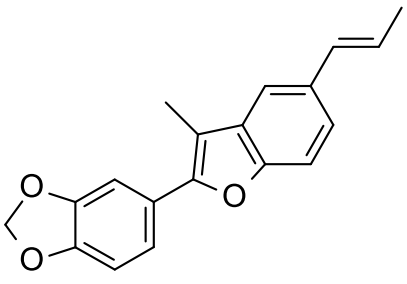
An antibacterial metabolite known as hypericin (**35**) has been isolated from St John's Wort (*Hypericum perforatum*), a herb widely used in the Western Herbal Medicine for the treatment of depression. This compound exhibited highly promising antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and penicillin-resistant variants reported with MICs of 0.1 μg/mL.[73] Medicinal Phytochemistry group at the UCL School of Pharmacy led by Professor Simon Gibbons[74] carried out extensive bioassay directed investigation on the genus *Hypericum* to explore antibacterial compounds with potential activity against a number of clinical isolates of methicillin resistant *S. aureus* (MRSA) strains. Bioassay-guided investigations into several *Hypericum* species led to the isolation of new acylphloroglucinols,[74-76] nor-lignans[77] and xanthone[78] with significant activity against *S. aureus*. Hyperenone A (**36**), a constituent of *H. acmosepalum*,[74] showed antibacterial activity against *Staphylococcus aureus* and *M. tuberculosis* as well as the inhibition of the adenosine triphosphate-dependent MurE ligase of *M. tuberculosis*, a crucial enzyme for peptidoglycan biosynthesis. An acylphloglucinol, (*S*,*E*)-1-(2-((3,7-dimethylocta-2,6-dien-1-yl)oxy)-4,6-dihydroxyphenyl)-2-methylbutan-1-one (trivial name, olympicin A) was isolated from *H. Olympicum* with promising (MICs of 1 μg/mL) activity against a panel of clinical isolates of multidrug-resistant (MDR) and methicillin resistant *Staphylococcus aureus*.[73] Such high antibacterial activity inspired the group to carry out the total synthesis of olympicin A as well as to make its analogues to fit into structure activity relationship (SAR) study. Olympicin A was synthesized in large scale and a number of its analogues were prepared by simply modifying two substituents. Among the analogues, the synthetic compound prepared by substituting gerenyl side chain with octyl group revealed same or 2-fold activity compared to natural olympicin A.[79]



Like phloroglucinols, the antimicrobial activities of phenolic compounds have also been extensively studied. For example, eupomatenoid-3 (**37**), eupomatenoid-5 (**38**), eupomatenoid-6 (**39**) and conocarpan (**40**), isolated from *Piper regnellii* showed antibacterial activity. Among them, compounds **38** and **39** exhibited significant antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *B. subtilis* with MICs of 1.56- 6.25 µg/mL.[80] Similarly, several antimicrobial isoflavanones including 5,7-dihydroxy-2'- methoxy-3',4'-methylenedioxyisoflavanone and 4',5-dihydroxy-2',3'-dimethoxy-7-(5-hydroxyoxychromen-7yl)-isoflavanone were reported from a perennial herb, *Uraria picta* Desv., which has been traditionally used as an antidote to the venom of a dangerous Indian snake, *Echis carinata*.[81] These isoflavanones showed the antimicrobial activity against bacteria and fungi with MICs in the range of 12.5-100 µg/mL. [81] *Flemingia paniculata* several antimicrobial compounds including a simple salicylic acid derivative, 2-carboxy-3-(2-hydroxypropanyl)phenol,3-hydroxy-4-methoxycinnamaldehyde and several isoflavones including 5,7,4'-trihydroxy-8-(1,1-dimethyl-prop-2-enyl)-isoflavone, 5,7,2',4'-tetrahydroxy-8-(1,1-dimethyl-prop-2-enyl)-isoflavone and 5,2',4'-trihydroxy-4",4",5"(ζ)-trimethyl-4",5"-dihydrofurano-(7,6,2",3")-isoflavone[82] with significant activities (MICs 1.57-200 μg/mL).[83] The highest potency (MIC 1.57 μg/ml; 0.005 mmol) was exhibited by 5,7,4'-trihydroxy-8-(1,1-dimethyl- prop-2-enyl)-isoflavone (**41**), against *S. aureus*. [83]

Quinones are well documented for their biological properties including antimicrobial activity. Bouldiaquinone, 2-acetylfuro-1,4-naphthoquinone, 2-hydroxy-3-methoxy-9,10-dioxo-9,10-dihydroanthracene-1-carbaldehyde and lapachol (**42**) were reported from the root bark of *Newbouldia laevis* with broad-spectrum *in vitro* antimicrobial activity against six Gram-positive and twelve Gram-negative bacterial species, as well as *Candida* strains with MIC values in the range 0.076–9.76 μg/mL.[84] Aerial part of *Saprosma fragrans* was reported to produce 3,4-dihydroxy-1-methoxyanthraquinone-2-carboxaldehyde and damnacanthal (**43**),

which exhibited antifungal activities against *Trichophyton mentagrophytes*, *Cryptococcus neoformans*, *Sporothrix schenckii* and *T. mentagrophytes* (MIC = 1.56-12.5 μg/mL).[85]



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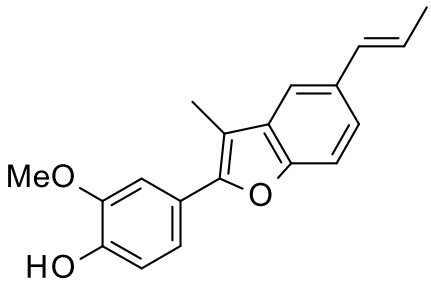
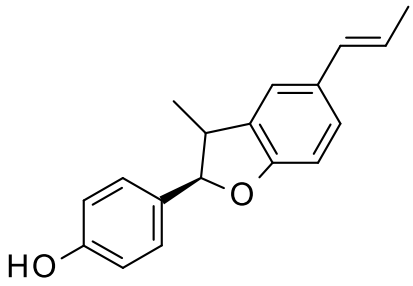


38 R = OMe
39 R = H



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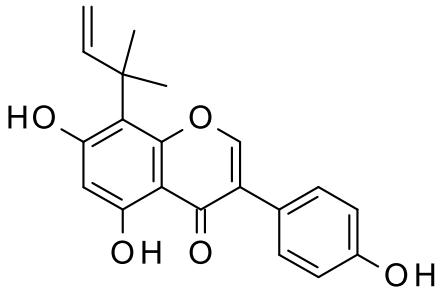

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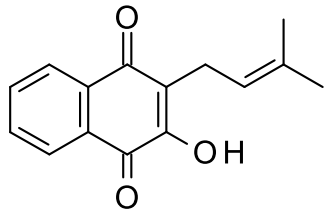

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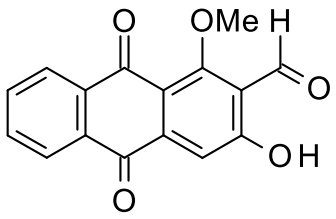

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Many plant extracts containing steroidal saponins possess antimicrobial propery. For example, the flower of *Allium leucanthum* produced aginoside and (25*R*)-5α-spirostan-3β,6β-diol-3-*O*-{β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)- β-D-galactopyranoside}, which showed *in vitro* antifungal activity against seven *Candida* strains with MFCs in the range of 6.25-12.5 μg/mL.[86] Tigogenin 3-*O*-β-D-xylopyranosyl-(1→2)-[β-D-xylopyranosyl(1→3)]-β-D-glucopyranosyl-(1→4)-[α-L-rhamnopyranosyl-(1→2)]-β-D-galactopyranoside and tigogenin 3-*O*-β-D-glucopyranosyl-

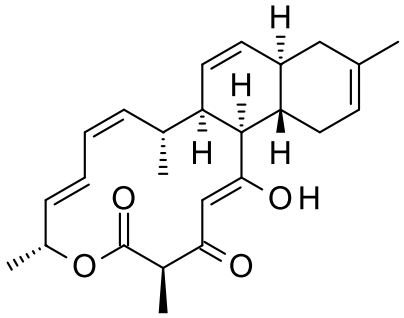
(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside were isolated from *Tribulus terrestris*, and showed significant activity against *C. albicans* (MIC = 10-2.3 μg/mL) and *Cryptococcus neoformans* (MIC = 1.7-6.7 μg/mL).[87] Another steroidal saponin, dioscin, isolated from the rhizomes of *Smilacina atropurpurea*, showed antifungal against *C. albicans* and *C. glabrata* (MFCs ≤5.0 μg/mL).[88]

These are just a few examples of plant-derived antimicrobial compounds from several that have been reported in recent years. However, their *in vitro* antimicrobial efficacy is nowhere near any well-known antimicrobial agents obtained from microbial sources, and none of these compounds have been tested *in vivo*.

3.3 Antimicrobial natural products from marine organisms

The sections above have documented some examples of antimicrobial compounds predominantly from terrestrial organisms. However, various organisms from marine origin, have recently been shown as potential sources of new compounds with various therapeutic applications, including eeficacy against infections. During last few decades there have been an incredible amount of research carried out on marine organisms and phytoplankton to explore the bioactive compounds to be considered as lead compounds in drug discovery including antimicrobial compounds. The marine environment is a rich source for unique actinomycete bacteria, which have produced thousands of metabolites with significant biological activity.[89] Many of the marine-derived actinomycetes have been produced structurally diverse secondary metabolites with promising anticancer properties.[90] With regards to the discovery of antibiotics, marine actinomycetes are less developed, however the isolation of promising antibiotics such as anthracimycin has inspired natural product chemists to explore this source further.[91] Anthracimycin (**44**) is a polyketide antibiotic isolated first in 2013 from a marine actinomycete of the genus *Streptomyces* (strain CNH365) collected off the shore of Santa Barbara at USA.[91] Another strain of *Streptomyces* (strain T676) isolated off the coast of St. John's Island, Singapore, also produced anthracimycin.[92] However, the research on marine actinomycetes for bioactive lead compounds started far earlier. Five structurally unique depsipeptides, salinamides A-E, were reported from a marine-derived *Streptomyces* sp. (strain CNB-091), which showed significant antibacterial and anti-inflammatory properties.[93] Subsequently, salinamide A exhibited significant inhibitory activity against RNA polymerase (RNAP) from Gram-positive and Gram-negative bacteria.[94] Further research was carried out on the *Streptomyces* sp. CNB-091 resulting in the isolation of salinamide F, from the ethyl acetate fraction, which also revealed significant RNAP-inhibitory

activity against *S. aureus* (IC_{50} 4 µM) and *E. coli* (IC_{50} 4 µM) as well as antibacterial activity against *Enterococcus faecalis* (MIC 12.5 µg/mL), *S. aureus* (MIC100 µg/mL), *H. influenzae* (MIC 12.5 µg/mL), *Neisseria gonorrhoeae* (MIC 25 µg/mL), *Enterobacter cloacae* (MIC 50 µg/mL) and *E. coli* (MIC 0.20 µg/mL).[95]



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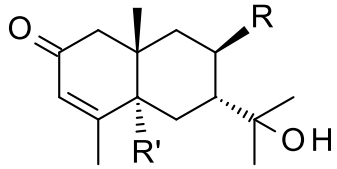
Cyclic depsipeptides, ulleungamides A and B,[96] were isolated from cultures of *Streptomyces* sp. which showed limited activity against *Staphylococcus aureus* and *Salmonella typhimurium* but no cytotoxicity. The antibacterial activity was tested using disc diffusion method. Zones of inhibitions of these two compounds against the above two organisms were found in the range of 9-16 mm compared to 20-25 mm of a positive standard, tetracycline. Eeudesmene-type sesquiterpenes, kandenols A-E (**45-49**) [97] from the culture broth of *Streptomyces* sp. (strain HKI0595) showed weak to moderate antimicrobial activities against *Bacillus subtilis* ATCC 6633 and *Mycobacterium vaccae*.[97]

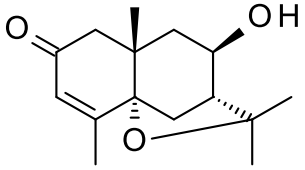


45 R = OH, R' = H

46 R = OH, R' = OH

47 R = OH, R' = O-OH

48 R = H, R' = O-OH



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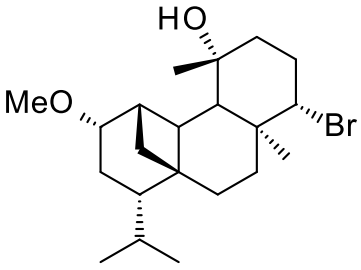

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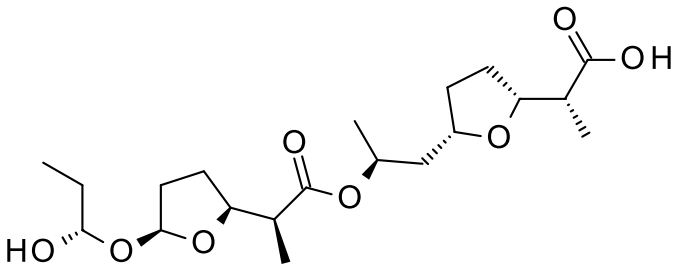
Naturally occurring brominated diterpenes with tetracyclic skeletons, ioniols I (**50**) and II,[98-100] were isolated from *Sphaerococcus coronopifolius*, a marine sponge collected from the rocky coasts of Corfu island in the Ionian Sea. These metabolites were evaluated for their antibacterial activity against a panel of clinical isolates of multidrug-resistant (MDR) and methicillin-resistant *Staphylococcus aureus* (MICs 0.5-128 μg/mL). Novel C_{15} eight-membered cyclic ethers[101] with a characteristic terminal *cis* eneeyne moiety were isolated from the red alga, *Laurencia glandulifera*, collected from the Crete island in South Greece. These compounds exhibited antibacterial activity against MRSA strains with MICs of 8-256 μg/mL.[101] A total of 17 diterpenes[102] featuring the dolabellane skeleton were isolated from the organic extracts of the brown alga, *Dilophus spiralis*. Some of these showed good antibacterial activity against six strains of *S. aureus*, including multidrug-resistant and methicillin-resistant variants.[102]

Neomaclafungins A-I[103], 26-membered macrolides of the oligomycin subfamily, were isolated from *Actinoalloteichus* sp. NPS702, a marine sediment collected from USA Bay, Kochi Prefecture, Japan. These macrolides exhibited significant antifungal activity *in vitro* against *Trichophyton mentagrophytes* (ATCC 9533) with MICs of 1-3 μg/mL.[103]

Polycyclic secondary metabolites, citreamicins A and B, citreaglycon A and dehydrocitreaglycon A,[104] isolated from marine-derived *Streptomyces caelestis* exhibited antibacterial activity against *Staphylococcus haemolyticus*, *S. aureus*, and *B. subtillis*. Citreamicin A, citreamicin B, and citreaglycon A revealed strong activities against MRSA ATCC 43300 with MIC values of 0.25, 0.25, and 8.0 μg/mL, respectively.[104] Isorhodoptilometrin-1-methyl ether, emodin, 1-methyl emodin, siderin, arugosin C, and variculanol obtained from the marine fungus *A. versicolor* were reported to exhibit antimicrobial activity, anticancer activity, and inhibition of Hepatitis C virus protease.[105] A marine-derived fungus *Nigrospora*, produced anthraquinone derivative, 3-acetoxy-4-deoxybostrycin, which exhibited promising activity against *Bacillus cereus* (MIC 48.8 nM).[106] C-glycosylated benz[α]anthraquinone derivatives, urdamycinone E, urdamycinone G, dehydroxya-quayamycin, isolated from the marine *Streptomycetes* sp. displayedpotent activity against *M. tuberculosis* with MICs of 3.13-12.50 μg/mL.[107]

Trichodermaquinone and trichodermaxanthone from the marine-derived fungus *Trichoderma aureoviride* PSU-F95 demonstrated significant antibacterial activity against MRSA with MIC values of 8 and μg/mL, respectively.[108] Alkanoyl imidazoles, bulbimidazoles A-C,[109] isolated from the culture extract of the gammaproteobacterium *Microbulbifer* sp. DC3-6 collected from a stony coral of the genus *Tubastraea* displayed broad-

spectrum antimicrobial activity (MICs ranging from 0.78 to 12.5 μg/mL).[109] Microketides A and B, a pair of C-11 epimeric polyketides, from the gorgonian-derived fungus *Microsphaeropsis* sp. RA10-14 collected from the South China Sea showed strong activity against *Pseudomonas aeruginosa*, *Nocardia brasiliensis*, *Kocuria rhizophila*, and *Bacillus anthraci* with the same MIC value as ciprofloxacin (0.19 μg/mL).[110] The liquid culture of a *Streptomyces* sp. (strain BD21-2) collected from a shallow-water sediment sample from Kailua Beach, Oahu, Hawaii produced bonactin (**51**), which showed antimicrobial activity against both Gram-positive and Gram-negative bacterial as well as fungal strains.[111]



51

3.4 Antimicrobial natural products from animals

Animals have also been reported to secrete compounds of biological interests including those with significant antimicrobial properties. These animals include amphibians species, mammals and similar animals capable of producing metabolites with promising biological activities. The skin glands of amphibians species have been documented to produce antimicrobial peptides (AMPs) that are crucial in the first line of defence against microbial invasion. The vast majority of AMPs isolated from the frog skin are reported to exert potent activity against antibiotic-resistant bacteria, protozoa, yeasts, and fungi by permeating and destroying the plasma membrane and inactivating intracellular targets. Importantly, since they do not bind to a specific receptor, AMPs are less likely to induce resistance mechanisms. Although most of the AMPs have common characteristics, they differ in amino acids sequences which produce a wide range bioactivity with varying degrees of efficacy.

European fire-bellied toad, *Bombina* sps., is known to produce skin secretions with peptides, such as bombinins and bombinins H, with potential antimicrobial activity.[112] Bombinins and bombinins H, which are quite large peptides, were isolated from common precursors containing one or two bombinin copies at the amino and a single bombinin H at the carboxyl end. Bombinins have showed activity against Gram-positive and Gram-negative bacteria and fungi but virtually inactive in haemolysis assays. However, bombinins H showed

lower bactericidal activities but lyse erythrocytes.[112] Bombinins, identified from the ancient toad belonging to the genus *Bombina*, are a group of amphibian-derived peptides with broad-spectrum antimicrobial activities. A novel bombinin precursor encoded a bombinin-like peptide (BLP-7) and a novel bombinin H-type peptide (Bombinin H-BO) were identified from the skin secretion of Oriental fire-bellied toad, *Bombina orientalis*.[113] In the antimicrobial experiment, the synthetic replicate of BLP-7 exhibited more potency than Bombinin H-BO against Gram-positive and Gram-negative bacteria and yeast.

A total of 11 AMPs was reported from *Pleurodema somuncurence* (Anura: Leptodactylidae: Leiuperinae). Three [Somuncurin-1 (FIIWPLRYRK), somuncurin-2 (FILKRSYPQYY), and thaulin-3 (NLVGSLLGGILKK)][114] inhibited the growth of *Escherichia coli* but only Somuncurin-1 showed antibacterial activity against *Staphylococcus aureus*. In a biophysical membrane model, this peptide showed a greater permeation effect in prokaryotic-like membranes and capability to restructure liposomes, suggesting fusogenic activity, which ultimate could cause cell aggregation and disruption of cell morphology. Eight new peptides isolated from the skin secretion of the frog, *Leptodactylus pustulatus,*[115] revealed structural similarities between them and other antimicrobial peptides reported from the same genus. Among these peptides, ocellatins-PT1 to -PT5 (25 amino acid residues) are amidated at the C-terminus, whilst ocellatins-PT6 to -PT8 (32 amino acid residues) have free carboxylates. All peptides, except for ocellatin-PT2, showed antibacterial activity against at least one Gram-negative strain. Ocellatin-PT8 inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Salmonella choleraesuis* strains with MICs of 60-240 μM. An LC-MS-MS to analysis on the skin samples of *Hypsiboas pulchellus*, an Argentinian wild frog, has identified antimicrobial peptides with molecular mass within 1000-2000 Da.[116] Out of 23 novel sequences identified by MS three (named P1-Hp-1971, P2-Hp-1935, and P3-Hp-1891) were synthesised. These three AMPs inhibited the growth of Gram-positive *Staphylococcus aureus* (MICs of P1-Hp-1971, P2-Hp-1935, and P3-Hp-1891: 8, 66, and 17 μM, respectively) and Gram-negative *E. coli* (MICs of P1-Hp-1971, P2-Hp-1935, and P3-Hp-1891: 16, 33, and 17 μM, respectively) revealing that P1-Hp-1971 and P3-Hp-1891 were the most active peptides.[116]

Chitosan, a polycationic biopolymer naturally present in the exoskeletons of crustaceans and arthropods, has been used as food preservative because of their ability to suppress fungal colony growth and inhibit fungal spore germination at a 0.01% (w/v) concentration. [117] Its antibacterial activity evaluated against several Gram-negative (*E. coli*, *P. fluorescens*, *S. Typhimurium*, *Vibrio parahaemolyticus*) and Gram-positive bacteria (*L. monocytogenes*, *Bacillus megaterium*, *B. cereus*, *S. aureus*) [118] revealed that chitosan

inhibited the growth of most of the tested bacteria showing stronger bactericidal effects against Gram- positive bacteria than Gram-negative bacteria at a concentration of 0.1% in agar medium. An investigation on the antimicrobial activity of a chitosan against various food poisoning and food spoilage bacteria revealed that the chitosan mixture retarded the growth of *Salmonella* spp. and reduced the population of *Staphylococcus* spp. in raw milk. [119] A study conducted to determine the shelf-life of oysters stored at 5±1°C established that chitosan at a concentration of 5.0 mg/mL extended the shelf-life of oysters from 8-9 days to 14-15 days which indicated the great potential of chitosan for seafood preservation.[120]

Lactoferrin, an iron-binding glycoprotein present in the milk of human and mammals, has shown to have significant antimicrobial activity against a wide range of bacteria and viruses.[121] Its antimicrobial activity has been reported against foodborne microorganisms including *Carnobacterium*, *L. monocytogenes*, *E. coli*, and *Klebsiella*[122] with a reduction of 4 log CFU/mL of *Cronobacter* species in the presence of 2.5 mg/mL lactoferrin in 0.2% peptone water within 4 h incubation at 37°C. In combination with nisin (0.1 mg/g), lactoferrin (0.2 mg/g) displayed a significant reduction in spoilage bacterial counts (total aerobic bacteria, coli- form, *E. coli*, total psychrophilic bacteria, *Pseudomonas* species, yeast and moulds) and extend shelf-life of up to 10 days in meatballs.[123] Milk-derived casein and whey proteins are known to possess various biological activities including antimicrobial activities.[124,125] Casocidin, a peptide produced by hydrolysis of aS2-casein by chymosin, revealed antibacterial activity against *Staphylococcus* spp., *Sarcina* spp., *B. subtilis*, *Diplococcus pneumoniae*, and *Streptococcus pyogenes*.[124] Isracidin, another casein derived peptide, showed antibacterial activity against *S. aureus*, *L. monocytogenes*, and *C. albicans*.[125] Peptides such as casein A and B at 0.05 mM and 0.22 mM respectively, were shown to inhibit the growth of pathogenic *Enterobacter sakazakii*. [126] Peptides generated from aS2- casein, aS1-casein, and k-casein have shown antibacterial effects against *E. coli* and *B. subtilis*.[127] The whey protein of bovine milk is composed of mainly of β- lactoglobulin and α-lactalbumin. The peptides released during the digestion of β-lactoglobulin with trypsin has shown to possess antimicrobial activity against food- borne pathogens such as *S. aureus*, *L. monocytogenes*, *Salmonella* spp. and *E. coli* O157 at concentration of 10-20 mg/mL. [124,128]

4. Antimicrobial compounds in preclinical and clinical trials

There are over 50 new antibiotics which are currently at different stages in clinical trials with the potential to treat serious bacterial infections including those which developed resistance to the existing antibiotics. A majority of these are of synthetic and/ or semi-synthetic origin. Among the antimicrobial agents originated from natural sources, which are undergoing

preclinical and clinical development stages are mostly antimicrobial peptides (AMPs)[129] and are usually derived from microorganisms or animal sources. Some of these AMPs with potential antimicrobial activity and proven mechanism actions have been outlined below.

MU1140, a lantibiotic peptide isolated from *Streptococcus mutans*, is currently undertaking preclinical development by Oragenics, Inc. It has been proven to exert activity against all gram-positive bacteria, especially methicillin-resistant *S. aureus* (MRSA). It acts through not only membrane disruption, but also inhibition of cell wall biosynthesis.[130]

Arenicin (AP139) isolated from lugworm *Arenicola marina* consists of 21-residue peptide, which has a positively charged amphipathic β-hairpin structure linked with one disulfide bond. This is undergoing through preclinical trial by Adenium Biotech. It reveals bactericidal activity against multidrug-resistant gram-negative bacterial infections by membrane pore formation. [131]

EA-230, developed by Exponential Biotherapies, is a linear tetrapeptide derived from the β-chain of the human chorionic gonadotropin hormone (β-hCG). The mode of action is known to be immunomodulation, such as the release of pro-inflammatory cytokines and a reduction of neutrophil influx. It has demonstrated anti-inflammatory activity when injected intravenously. Clinical trials for acute systemic inflammation (e.g., sepsis) and inflammation caused by organ dysfunction (e.g., acute kidney injury) are undergoing.[132]

PAC113, developed by Pacgen Biopharmaceuticals, is a 12-amino acid linear peptide derived from histatin 5, a human salivary α-helical peptide. It exerts antimicrobial activity via membrane disruption, as well as immunomodulation. The phase II clinical trial has been completed treatment for oral candidiasis in HIV patients through mouth rinse.[133]

Novexatin (NP213), developed by Novabiotics, is a cyclic cationic peptide consisting of seven arginine residues. It is going through the phase IIb clinical trial for the treatment of fungal nail infections with topical administration. It exerts its action through membrane disruption.[133]

PXL01, a macrocyclic peptide comprised of 25 amino acids connected through a disulfide bond, is derived from human lactoferrin which is currently being evaluated by ProMore Pharma. It showed antimicrobial activity for the topical treatment of postsurgical adhesions and scar prevention. It acts through an immunomodulatory mechanism (e.g., inhibition of the release of pro-inflammatory cytokines).[132]

Ramoplanin (NTI-851), developed by Nano-therapeutics, is a macrocyclic glycolipodepsipeptide produced by *Actinoplanes*spp. It reveals bactericidal activity by blocking the cell wall peptidoglycan synthesis of gram-positive bacteria. Its phase III clinical study of the peptide was initiated for the oral treatment of vancomycin-resistant *enterococcus* (VRE) colonization and also its phase II trial against *Clostridium difficile*.[134]

5. Conclusions

Nature continues to produce metabolites of chemical diversity with a large array of potential biological activities, which have contributed significantly in drug discovery including the development of new antibiotics. A vast majority of today's antibiotics available for clinical uses are either derived directly from natural sources or their semisynthetic analogues. However, the global crisis of antimicrobial resistance urges the development of new antibiotics, which could fight against the so-called 'superbugs' escaping the lethal actions of existing antibiotics. Such superbugs raising alarming situation of AMR have been grouped together and acronymically dubbed as ESKAPE pathogens including multi-drug resistance ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumonia,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa and* ***E****nterobacter* species.[135] These ESKAPE pathogens require immediate attention of antimicrobial drug discovery groups around the world to find new and safe antibiotics in order to control the situation of global healthcare threat. Collaborative research approaches among both academic and industrial scientists including natural product chemists, medicinal chemists, microbiologist and biotechnologists are also much needed.

Besides the discovery of new antibiotics, it is the responsibility of healthcare professionals and general public to preserve the efficacy of currently available antibiotics by raising public awareness on the appropriate uses of antibiotics. Although there has been a significant amount of work carried out through antibiotics stewardship, further work is necessary to encourage the reduction of over prescriptions and misuses of antibiotics. AMR situation is even much worse in the developing countries, where such situation could be improved by educating the general public for appropriate uses of antibiotics and also alerting the physicians to be more careful in prescribing antibiotics.

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