Actinomycetes: a yet inexhaustive source of bioactive secondary metabolites

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The rapid emergence of antimicrobial resistance among pathogens has led to a renewed interest to search for novel antimicrobial agents. The history of new drug discovery processes shows that novel skeletons have, in the majority of cases come from natural sources. This involves screening of microorganisms or plant extracts. They have been the source of, or inspiration for the development of chemical entities introduced as pharmaceutical. Among microorganisms, actinomycetes are enthralling resource due to their ability to produce novel bioactive secondary metabolites with antimicrobial activities. They have proven to be an inexhaustive mine of antimicrobial agents, especially those potent against pathogenic organisms. Microbial secondary metabolites, especially those from actinomycetes have been a phenomenal success for the discovery of novel drugs. They produce a wide range of secondary metabolites and more than 70% of the naturally derived antibiotics are currently in clinical use. They remain a fundamental source of new chemical diversity and an important part of drug discovery. Their ingenuity and immense industrial value is extremely noteworthy. The discovery of Streptomycin from actinomycetes has been imperative to the exploration of this group as a source of novel bioactive compounds. This group of organisms have produce antibiotics in different classes such as aminoglycosides, ansamycins, anthracyclines, glycopeptides, β-lactams, macrolides, nucleosides, peptides, polyenes, polyethers, and tetracyclines. Existence of actinomycetes has been reported in both terrestrial and marine habitats. This chapter highlight the bioactive metabolites produces by actinomycetes and their mode of action.

Keywords actinomycetes; antimicrobial agents; secondary metabolites, mechanism of action

1. Introduction

History had shown that discovery of novel antimicrobial agents have often times come from natural sources [1, 2]. These natural products having novel skeletons have been found to possess important biological activities and producing a significant number of therapeutic agents in clinical all around the world. They even serve as template for the synthesis of synthetic and semi-synthetic drugs. These discoveries involve the screening of microorganisms and plants from nature, using various techniques [3]. Microorganisms over the years have proved to be fascinating sources of natural products for the industries especially pharmaceutical industries [4]. The importance of microorganisms' sources for the discovery of novel natural products with a pharmaceutical potential has been proved fruitful during the last decade and was highlighted in various excellent review articles. Microbial natural products are noteworthy for their high therapeutic index and desirable pharmacological activities.

Most of the microbial bioactive compounds discovered so far originated from actinomycetes accounting for about two-third of antibiotics, including those in clinical uses. Actinomycetes are the most economically and biotechnologically worthwhile microorganisms [5-7]. They have produced a wide range of secondary metabolites of various medical importances such as antibiotics, antifungal, antiprotozoal, antiviral, anticholesterol, antihelminth, anticancer, and immunosuppressant. Among the 140 described Actinomycetes genera, only a few are responsible for the over 10,000 bioactive compounds in clinical use.

Actinomycetes are Gram-positive bacteria of the order actinomycetales; they are characterized by filamentous morphology, DNA with a high in G+C content presence of LL-Diaminopimelic acid (LL-DAP) and the presence or absence of characteristic sugars in the cell wall. Actinomycetes are ubiquitous and form a stable and persistent population in various ecosystems [8, 9]. The discovery of new actinomycete taxa from diverse habitat with unique metabolic activity often led to the discovery of novel antimicrobial agent. Various antimicrobial agents have been isolated and characterized from actinomycetes including aminoglycosides, anthracyclines, glycopeptides, macrolides, beta-lactams, polyenes, phenazine, and tetracyclines. In this chapter, we point out biological activities of secondary metabolites produced by actinomycetes in effort to combat infectious diseases.

2. Actinomycetes as producers of secondary metabolites

Microbial secondary metabolites have been in the frontier in the discovery of novel antimicrobial agents for pharmaceutical industry, and today all evidence suggests that novel compounds with potential therapeutic applications are still waiting to be discovered from secondary metabolites especially those produced by actinomycetes. Actinomycetes are prolific producers of secondary metabolites with biological activities [10]. Secondary metabolites

are metabolic products that are not essential for vegetative growth of the producing organisms but they are considered differentiation compounds conferring adaptive roles, for example, by functioning as defense compounds or signalling molecules in ecological interactions. They are produced at the end of the exponential growth phase and their syntheses greatly depend on the growth conditions. Production is usually when growth is limited by the exhaustion of one key nutrient such as carbon or nitrogen [11, 12]. They are structurally diverse and most of them are endowed with biological activities, such as antimicrobial agents, toxins, pesticides, ionophores, bioregulators, and quorum signalling. These bioactive metabolites are profoundly used as antimicrobial agents for the treatment of diverse ailments [13].

Secondary metabolites usually comprise various chemical moieties, such as polyketide backbones, amino acid derivatives and sugars. Biosynthesis of secondary metabolite is catalysed by a number of enzymes, usually encoded by genes. These genes occur adjacent to one another in cluster. The gene cluster contains all the necessary genes for the synthesis of a particular secondary metabolite. This includes: the genes that encode the biosynthetic enzymes, regulatory proteins, genes for resistance to the toxic action of secondary metabolites and genes for secretion of the metabolites. Enzymes such as synthase (PKS) and non-ribosomal peptide synthetase (NRPS) are involved in the synthesis of secondary metabolites [14]. Other enzymes responsible for the synthesis of other constitutive compounds, such as sugars, are often encoded by genes adjacent to the gene cluster. Through processes such as elongation, synthesis, glycosylation, alkylation and oxidation, structurally diverse and complex metabolites are produced. The whole process of production and transportation of secondary metabolites are strictly regulated by transcriptional regulators and transporters [15]. The genes encoding for tailoring enzymes, transcriptional regulators and transporters are often located adjacent to PKS and NRPS genes. The size of the gene cluster responsible for the synthesis of each secondary metabolite is usually between 10 -100 kb.

Previous studies showed that the gene cluster responsible for the production of secondary metabolites is not found in all bacteria and even in those present it is not uniformly distributed among them. For example *Streptomyces coelicolor* possesses more than 20 gene clusters while *S. avermitilis* possesses 30 gene clusters for the synthesis of secondary metabolites [16]. Genome mining for new candidate secondary metabolic pathways based on clustering and co-expression has proved to be a highly successful approach in microbes [17]. This helps to predict the types of antibiotic one might expect to find after extraction and purification. With the growing number of genome nucleotide sequence information in the GenBank and the advent of next generation sequencing it will be possible to search for candidate secondary metabolite gene cluster in a wide range of actinomycetes species. The evolution of microbial natural product collections and development of high-throughput screening methods have attracted researchers to the use of natural product libraries in drug discoveries. Ecopia Biosciences Inc used high-throughput genome scanning to detect secondary metabolite gene clusters, then used the signature sequences to predict the structures [18]. Actinomycetes continue to be a productive and successful focus for natural products research, with many novel compounds been of eminent pharmacological valuable.

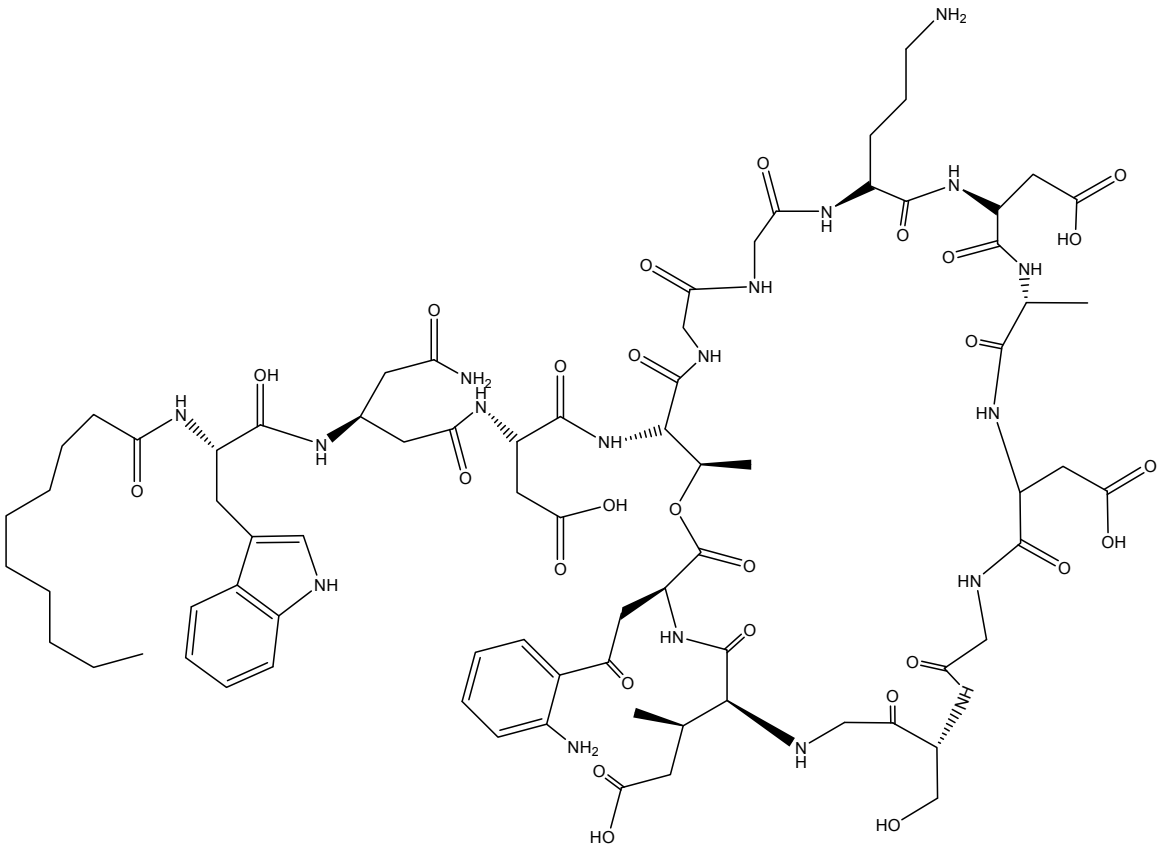
3. Antimicrobial activities of actinomycete secondary metabolites

Secondary metabolites produced by actinomycetes exhibit a great number of diverse and versatile biological effects, first of all antimicrobial activities. The order actinomycetales are renowned producer of bioactive metabolites with a track record of over 10,000 antimicrobial agents in clinical use [19]. The secondary metabolites produced by actinomycetes reveal multifarious biological activities such as antibacterial, antifungal, antiviral, anticancer, antiprotozoal, anticholesterol, anti-ageing, antihelminth and immunosuppressant. This group of compounds forms a heterogeneous assemblage of biologically potent molecules with diverse structures and mechanisms of action. The discovery of antimicrobial agents from actinomycetes led to a breakthrough in the world of medicine, due to their tremendous contribution in saving human from infectious diseases. Most bacteria infectious with no cure in the 19th century can easily be cure now with a short course of antibiotics, for example tuberculosis [20]. About 75% of the antibacterials are produced by actinomycetes. Many of these antibacterials exhibit broad spectrum of activities. The diversity in structure of these antibacterial is responsible for their broad spectrum antimicrobial activities and diverse mechanisms of action. They showed high potency against a large number of Gram-positive and Gram-negative organisms. Historically, actinomycetes have been the origin of the largest number of new antibiotic drug candidates and lead molecules with applications in many other therapeutic areas. This order alone produced 45% of the presently known bioactive microbial metabolites; over 10,000 compounds were still isolated from various actinomycetales species, 34% from *Streptomyces* and 11% from the non-*Streptomyces*. The most frequent producers, the *Streptomyces* species produces 7600 compounds (74% of all actinomycetales), while the rare actinomycetes represent 26%, altogether 2500 compounds. In this group *Micromonospora*, *Actinomadura*, *Streptoverticillium*, *Actinoplanes*, *Nocardia*, *Saccharopolyspora* and *Streptosporangium*

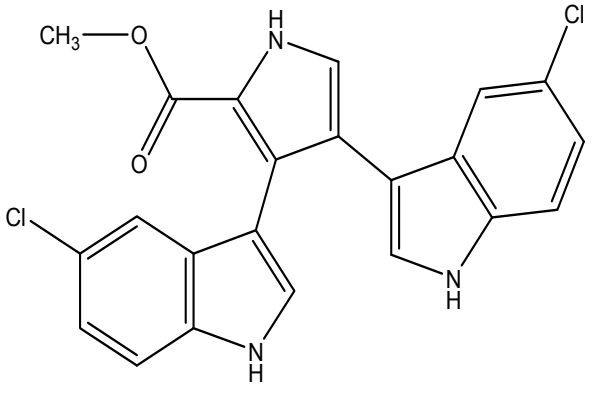
Daptomycin a cyclic lipopeptide antibiotic produced by *S. roseosporus* through NRPS mechanism is clinically used in the treatment of antibiotic resistant pathogens such as methicillin resistant *Staphylococcus aureus* and vancomycin-resistant enterococci. It exhibit bacteriocidal mode of action by causing rapid depolarization on the bacterial cell membrane, inhibition of biosynthesis of protein, cell wall. and lipoteichoic acid [21]. Lynamicins A-E are chlorinated bisindole pyrrole antibiotics produced by *Marinispora* sp. These alkaloids demonstrated strong broad spectrum

antimicrobial activities against drug-resistant pathogens [22]. Marinopyrroles A-F are alkaloids composed of two salicyloyl substituents on a 1, 3′-bipyrrole core. They are the first naturally occurring 1, 3′-bipyrrole reported and produced by *Streptomyces* spp isolated from marine sediment. These compounds show cytotoxicity against human cancer and antibacterial activities against resistant pathogens [23]. Echinomycin is a quinoxaline compound isolated from *S. echinatus*. This compound demonstrated antibacterial, antiviral and antitumor activities. [24, 25].

Platensimycin is a benzoic acid moiety antibiotic produced by *S. platensis*. Platensimycin exert its action by selectively inhibiting β-ketoacyl-acyl carrier protein (ACP) synthase II (FabF) in the synthetic pathway of fatty acids [26]. This compound is acknowledged as an effective broad-spectrum antibiotic against drug-resistant microorganism strains for example MRSA [27]. Diazepinomicin is a dibenzodiazepine alkaloid isolated from *Micromonospora* sp [28]. The compound show significant antitumor, antiparasitic, antioxidant and anti-protease activities [29]. The streptogramins are cyclic hexa or hepta despsipeptides antibiotics produced by the *Streptomyces* spp through NRPS mechanism. They are use in the treatment of infection caused by multiple drug-resistant pathogens. The mechanism of action is by inhibiting protein synthesis by preventing polypeptide elongation [30]. Spinosyns are glycosylated polyketide insecticide produce by *Saccharopolyspora species* through PKS mechanism. This compound is use as biocontrol for insect pest. They have a novel mechanism of action that involves disrupting the binding sites on nicotinic acetylcholine receptors of the insect nervous system. [31].



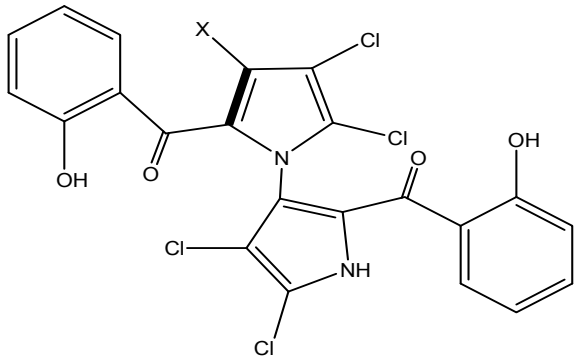
Daptomycin

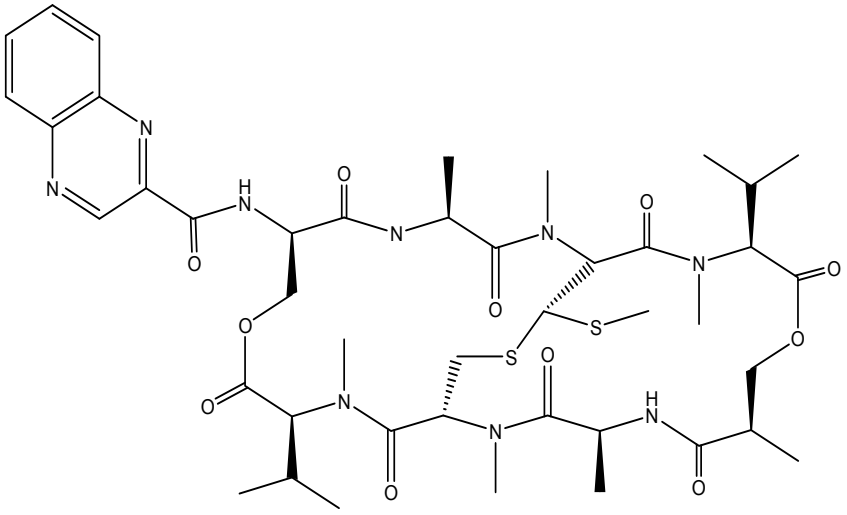


Lynamicin A

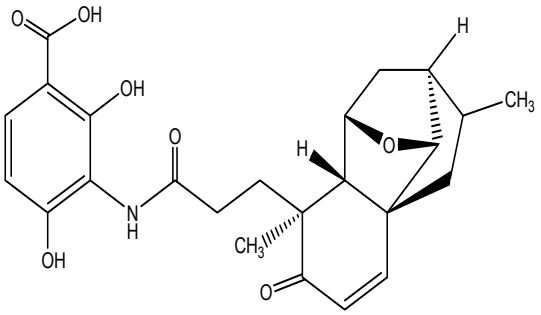
(-)-marinopyrrole A X=H

(-)-marinopyrrole B X = Br

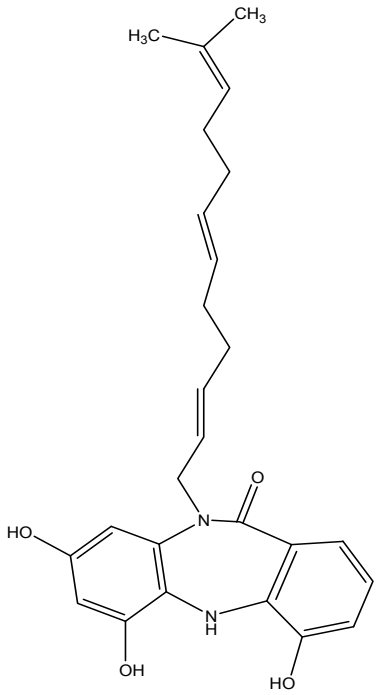




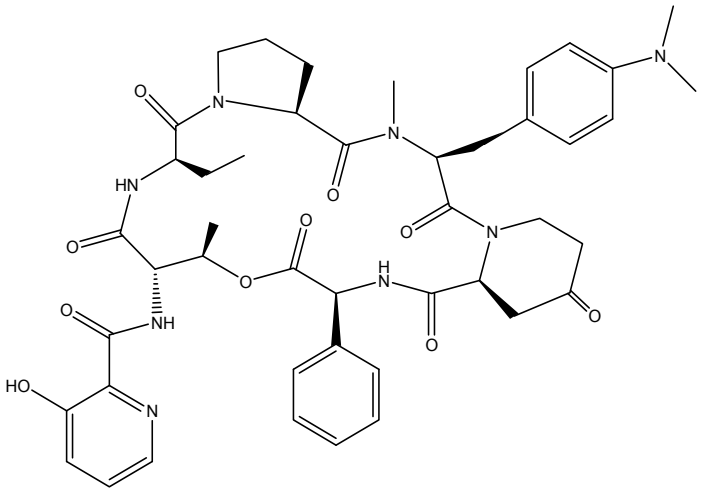
Echinomycin



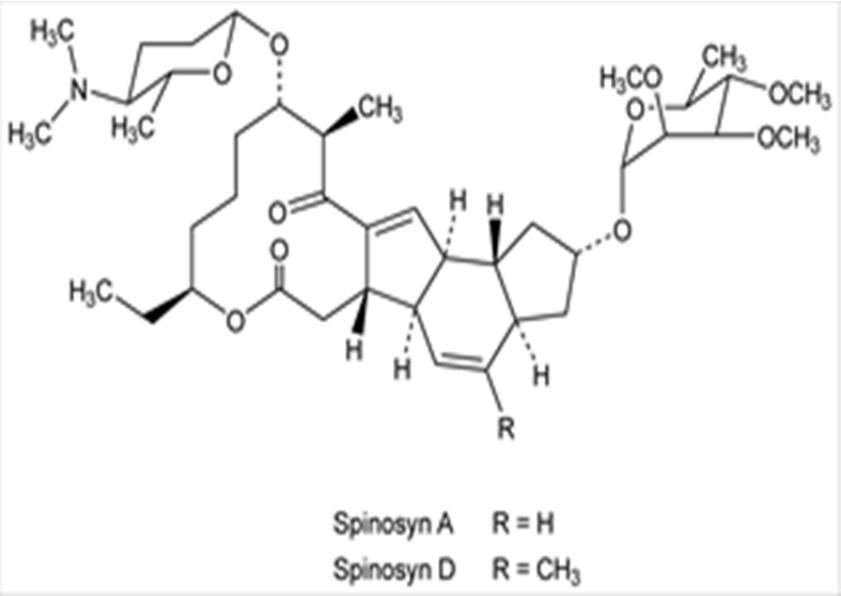
Platensimycin



Diazepinomicin



Streptogramins



Structures of various compounds produced by members of the order actinomycetales

4. Mechanism of actions of important classes of antibiotics produced by actinomycetes

Streptomyces, Micromonospora, Actinomadura, Amycolatopsis, Streptoverticillium, Actinoplanes, Nocardia, Saccharopolyspora Streptosporangium, Streptoalloteichus, Dactylosporangium, Frankia and Streptosporangium spp. are increasingly playing a significant role in the production of a wide range of antimicrobial metabolites of enormous important to the pharmaceutical industries. Important classes of antibiotics produced by actinomycetes include: β lactams, aminoglycosides, lipopeptides, glycopeptides, asamycins, anthracyclines, nucleosides, peptides, polyenes, polyethers, tetracyclines and macrolides.

4.1. β-Lactams

β-lactams are one of the most important classes of antibiotics in clinical use. This class include penicillins, cephalosporins, cephamycins, carbapenems, monobactams and clavulanic acid [32]. They are bacteriocidal in nature and exhibit a broader spectrum of antibiotic activities, which is active against both the Gram-positive and Gram-negative bacteria. The main producers of these antibiotics in the order actinomycetales are *Norcardia lactamdurans*, *Streptomyces clavuligerus, S. chartreusis, S. panayaensis, S. viridochromogenes, S. wadayamensis, S. jumonjinenesis,* and *S. limanii* [33].

Mechanism of action of β-lactams is by inhibiting the synthesis of the peptidoglycan layer in the bacterial cell wall especially the Gram-positive bacteria by blocking the action of transpeptidases. Transpeptidases also called penicillin-binding proteins (PBPs) are involved in the assembly of the bacterial cell wall. It is during this stage that linear strands of peptidoglycan are cross-linked into a fishnet-like polymer. D-alanyl-D-alanine, is the terminal amino acid residues on the precursor N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG)-peptide subunit of the nascent peptidoglycan layer. The structural similarity between β-lactam antibiotics and D-alanyl-D-alanine facilitates their binding to the active site of transpeptidases. The carboxylate or sulfonate group of the β-lactam reacts with the serine residue of the transpeptidase to give an acyl enzyme, an acylated enzyme is inactive [34]. This irreversible inhibition prevents the enzyme from carrying out transpeptidation of the nascent peptidoglycan layer. Inhibition of the transpeptidation by β-lactam causes a build-up of peptidoglycan precursors; this triggers the digestion of existing peptidoglycan by autolytic hydrolases without the formation of new peptidoglycan [35]. As a result, the action of the β-lactam antibiotics causes inhibition of cell wall synthesis. N-thiolated β-lactams have been found to possess antibacterial, anticancer and antifungal activities. This new member of β-lactams mechanism of action involves the inhibition of fatty acid biosynthesis especially in pathogens expressing high levels of coenzyme A [36].

4.2. Aminoglycosides

Aminoglycosides are one of the most important broad spectrum antibiotics in clinical use. These antibiotics are structurally similar with a core aminocyclitol moiety and various unusual sugars including aminosugars and deoxysugars [37]. They are classified into two groups depending on the structure of the aglycone. One group containing streptamine: originated from *myo*-inositol pathway and another group 2-deoxystretamine originated from 2-deoxy-*scyllo*-inosose pathway [38]. Aminoglycosides include important antibiotics such as streptomycin, neomycin, amikacin,

paromomycin, tobramycin, kanamycin, ribostamycin, nebramycin, apramycin, gentamicin, netilmicin, istamycin, sisomicin, lividomycin, spectinomycin, hygromycin, verdamicin, astromicin [39]. These antibiotics have been produced by *Streptomyces, Streptosporangium, Saccharopolyspora, Streptoalloteichus, Micromonospora, Dactylosporangium, and Frankia*.

Mechanism of action of aminoglycosides is by binding to the aminoacyl site of 16S rRNA within the 30S ribosomal subunit, leading to misreading of the genetic code and by inhibiting translocation of the tRNA-mRNA complex [40, 41]. The initial steps required for peptide synthesis are uninterrupted, such as binding of mRNA and the association of the 50S ribosomal subunit, but elongation fails to occur due to disruption of the mechanisms for ensuring translational accuracy by the antibiotic [42]. The irreversible binding of the antibiotic to the 30S bacterial ribosome inhibit the polypeptide chain elongation phase resulting in protein synthesis inhibition. It has also been found that some aminoglycosides prevent the transfer of the peptidyl tRNA from the A-site to the P-site, thus preventing the elongation of the polypeptide chain. Depending on concentration their effect can be bacteriostatic or bacteriocidal [39].

4.3. Glycopeptides

Glycopeptides are class of antibiotics composed of glycosylated cyclic or polycyclic non ribosomal peptides [43]. They are characterized by the presence of a unique long aliphatic chain attached to a sugar moiety. This class of antibiotics include vancomycin, teicoplanin, telavancin, bleomycin, ramoplanin, balhimycin, chloroeremomycin decaplanin, avoparcin, actinoidin. They are produced by *S. orientalis*, *S. candidus, S. toyocaensis, N. actinoides, Actinoplanes teichomyceticus, Amycolatopsis orientalis, Amy. balhimycina* [44]. The glycopeptide antibiotics are used as drugs of last resort to combat resistant Gram-positive pathogens, especially methicillin-resistant *Staphylococcus aureus* [45].

The mechanism of action of for glycopeptides is by inhibiting the maturation of the peptidoglycan layer in bacterial cell wall biosynthesis at the transglycosylation and transpeptidation steps [44]. The antibiotic binds to the amino acids within the cell wall preventing the addition of new units to the peptidoglycan layer. In particular, they bind to acyl-D-alanyl-D-alanine (D-Ala-D-Ala) terminus of lipid II. This interaction occurs through formation of five hydrogen bonds between the glycopeptide and the amide and carbonyl groups of the dipeptide moiety [46]. The antibiotic forms a tight complex with the lipid II intermediate; by shielding the D-Ala-D-Ala terminus, this prevents the subsequent action of the transpeptidase(s) (TP) and/or transglycosylase(s) (TG). The failure to form crosslinks between lipid II and the nascent PG chain lowers the rigidity of the cell wall and renders the bacterial cell susceptible to osmotic lysis [46].

4.4 Anthracyclines

Anthracyclines are among the most effective anticancer drugs in clinical use with widest spectrum of activity [47]. Their diversity is based on structural differences in the aglycone and on a wide array of attached sugar residues. This class of drugs include daunorubicin, doxorubicin, epirubicin, pirarubicin, idarubicin, valrubicin, nogalamycin, aclacinomycin, amrubicin and rhodomycin. They are produced by *Streptomyces* spp: *S. peuceticus, S. galilaeus, S. nogalater, S. purpurascens Micromonospora lupine.* These compounds are use in clinical treatment of a wide variety of cancers such as acute myeloid leukaemia, lymphomas, and a diversity of solid tumours including breast, small cell lung, cervical, head, and neck cancer [48].

Anthracycline has three mechanisms of action: (1) inhibits DNA and RNA synthesis by intercalating between base pairs of the DNA/RNA strand. In order for a cell to divide, the DNA in the cell's nucleus must be unravelled and then duplicated (transcription). Anthracyclines bind to portions of the unwound strand of nuclear DNA, halting the transcription process, thus preventing the replication of rapidly-growing cancer cells [49, 50]. (2) inhibits topoisomerase II enzyme, an enzyme that unzips the DNA molecule for replication. Anthracyclines interference with topoisomerase II by preventing the relaxing of supercoiled DNA, thus blocking DNA transcription and replication [51, 52]. (3) creates iron-mediated free oxygen radicals that damage the DNA, proteins and cell membranes [53].

4.5. Macrolides

Macrolides are class of antibiotics composed of a large macrocyclic lactone ring to which one or more deoxy sugars are attached. This class of antibiotics include erythromycin, clarithromycin, azithromycin, dirithromycin, roxithromycin, flurithromycin, josamycin, midecamycin, rokitamycin, miocamycin, spiramycin, rapamycin, telithromycin, pikromycin, [54]. They are produced by *Saccharopolyspora erythraea*, *S. hydroscopicus, S. venezuelae.* This class of compounds includes a variety of bioactive agents such as antibiotics, antifungal, prokinetics, and immunosuppressant [55]. The antimicrobial spectrum of macrolides includes activities against streptococci, enterococci, staphylococci and some pathogenic *Haemophilus influenzae*, *Neisseria* species, *Bordetella*, *Corynebacterium*, *Chlamydia*, *Mycoplasma*, *Rickettsia* and *Legionella* species [56].

The mechanism of action of macrolides is inhibition of bacterial protein biosynthesis. They reversibly bind to the 23S rRNA in the 50S subunit of the bacterial ribosome, and inhibit mRNA-directed protein synthesis. This action is carried out by preventing peptidyltransferase from adding the peptidyl attached to tRNA to the next amino acid, this also inhibits ribosomal translocation [57, 58]. Another mechanism is premature dissociation of the peptidyl-tRNA from the

ribosome. Macrolide antibiotics do so by binding reversibly to the P site on the subunit 50S of the bacterial ribosome [59]. Generally considered to be bacteriostatic, they may be bactericidal at higher doses.

4.6. Tetracyclines

Tetracyclines are antibiotics derivatives of polycyclic naphthacene carboxamide with octahydrotetracene-2-carboxamide skeleton. Examples of this class of antibiotics include tetracycline, demeclocycline, methacycline, minocycline, oxytetracycline rolitetracycline, lymecycline, and chlortetracycline [60]. They are produced by members of the genus *Streptomyces S. aureofaciens, S. rimosus*, and *S. viridofaciens*. They exhibited activity against a wide range of microorganisms including gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and protozoan parasites.

Tetracyclines mechanism of action is by preventing the attachment of charged aminoacyl-tRNA to the ribosomal acceptor (A) site on the ribosome [61]. Thus, they prevent introduction of new amino acids to the nascent peptide chain. They also simultaneously inhibit other steps of the protein biosynthesis. Tetracycline can also alter the cytoplasmic membrane and this in turn causes leakage of nucleotides and other compounds out of the cell [62]. Their action is bacteriostatic in nature.

4.7. Polyenes

Polyenes are antifungal agents with multiple conjugated double bonds. This class of antibiotics include amphotericin B, nystatin, natamycin, rimolidin, filipin, candicin, etruscomycin, hamycin and perimycin [63]. They are produced by the members of the genus *Streptomyces*: *S. nodosus, S. noursei, S. natalensis.* Polyenes have a broad spectrum of action and are useful in treating fungal infections such as candidosis, cryptococcosis, histoplasmosis, blastomycosis, paracoccidioidomycosis, coccidioidomycosis, aspergillosis, extracutaneous sporotrichosis, mucormycosis, hyalohyphomycosis and phaeohyphomycosis [64].

Mechanism of action of polyenes is by binding to ergosterol, the primary sterol in the fungal cell membrane. The consequence of this binding includes disruption of the osmotic integrity of the membrane, with leakage of intracellular potassium and magnesium, and also the disruption of oxidative enzymes in target cells [64, 65]. Polyenes act by inserting into the fungal membrane in close association with ergosterol. The subsequent formation of porin channels leads to loss of transmembrane potential and impaired cellular function. Bioactive secondary metabolites from actinomycetes have extensive application in clinical with different mechanism of actions. These bioactive compounds from actinomycetes are inexhaustible and only a fraction of these compounds have been identified so far. Discovering novel antimicrobial agents of clinical importance from actinomycetes is still a thrust area of research.

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References

[1] Chin Y, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. *American Association of Pharmaceutical Scientists Journal*. 2006;8:E239-E253.
[2] Ganesan A. The impact of natural products upon drug discovery. *Current Opinion in Chemical Biology*. 2008;12:306-317.
[3] Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *Journal of Natural Products*. 2012;75:311-335.
[4] Berdy J. Bioactive microbial metabolites. *Journal of Antibiotics*. 2005;58:1-26.
[5] Baltz RH. Antimicrobials from actinomycetes. Back to the future. *Microbe* 2007;2:125-131.
[6] Naine J, Srinivasan MV, Devi SC. Novel anticancer compounds from marine actinomycetes: A review. *Journal of Pharmarcy Research*. 2011;4:1285-1287.
[7] Raja A, Prabakarana P. Actinomycetes and drug-an overview. *Science Alert*. 2011;1:72-84.
[8] Adegboye MF, Babalola OO. Taxonomy and ecology of antibiotic producing actinomycetes. *African Journal of Agricultural Research*. 2012;7:2255-2261.
[9] Dietz A, Currie S, Jennie CH-C, Angela B. Chapter 5 - actinomycetes. Maintaining cultures for biotechnology and industry. San Diego: Academic Press; 1996. p. 85-99.
[10] Marinelli F, Marcone GL, *Microbial secondary metabolites. Comprehensive biotechnology* (second edition). Burlington, American: Academic Press; 2011. p. 285-297.
[11] Barrios-González J, Mejýa A. Production of antibiotics and other commercially valuable secondary metabolites. Current developments in solid-state fermentation. Heidelberg, New York: Springer; 2008. p. 302-336.
[12] Sanchez S, Demain AL. Metabolic regulation of fermentation processes. *Enzyme and Microbial Technology*. 2002;31:895-906.
[13] Vaishnav P, Demain AL. Unexpected applications of secondary metabolites. *Biotechnology Advances*. 2010;29:223-229.
[14] Donadio S, Monciardini P, Sosio M. Polyketide synthases and nonribosomal peptide synthetases: The emerging view from bacterial genomics. *Natural Product Reports*. 2007;24:1073-1109.
[15] Ichikawa N, Sasagawa M, Yamamoto M, Komaki H, et al. Dobiscuit: A database of secondary metabolite biosynthetic gene clusters. *Nucleic Acids Research*. 2013;41:D408-D414.

[16] Arias AA, Craig M, Fickers P. Science against microbial pathogens: Communicating current research and technological advances. In: Méndez Vilas A, editor. Gram-positive antibiotic biosynthetic clusters: a review. Badajoz, Spain: Formatex Research Center; 2011. p. 977-986.
[17] Osbourn A. Secondary metabolic gene clusters: Evolutionary toolkits for chemical innovation. *Trends in Genetics*. 2010;26:449-457.
[18] Baltz RH. Renaissance in antibacterial discovery from actinomycetes. *Current Opinion in Pharmacology*. 2008;8:557-563.
[19] Demain AL. Antibiotics: Natural products essential to human health. *Medicinal Research Reviews*. 2009;29:821-842.
[20] McDermott W, Muschenheim C, Hadley SJ, Bunn PA, et al. Streptomycin in the treatment of tuberculosis in humans. *Annals of Internal Medicine*. 1947;27:769-822.
[21] Baltz RH. Daptomycin: Mechanisms of action and resistance, and biosynthetic engineering. *Current Opinion in Chemical Biology*. 2009;13:144-151.
[22] McArthur KA, Mitchell SS, Tsueng G, Rheingold A, et al. Lynamicins a− e, chlorinated bisindole pyrrole antibiotics from a novel marine actinomycete. *Journal of Natural Products*. 2008;71:1732-1737.
[23] Hughes CC, Prieto-Davo A, Jensen PR, Fenical W. The marinopyrroles, antibiotics of an unprecedented structure class from a marine *streptomyces sp*. *Organic Letters*. 2008;10:629-631.
[24] Foster BJ, Clagett-Carr K, Shoemaker DD, Suffness M, et al. Echinomycin: The first bifunctional intercalating agent in clinical trials. *Investigational New Drugs*. 1985;3:403-410.
[25] Kong D, Park EJ, Stephen AG, Calvani M, et al. Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. *Cancer Research*. 2005;65:9047-9055.
[26] Wang J, Soisson SM, Young K, Shoop W, et al. Platensimycin is a selective fabf inhibitor with potent antibiotic properties. *Nature*. 2006;441:358-361.
[27] Singh SB, Jayasuriya H, Ondeyka JG, Herath KB, et al. Isolation, structure, and absolute stereochemistry of platensimycin, a broad spectrum antibiotic discovered using an antisense differential sensitivity strategy. *Journal of the American Chemical Society*. 2006;128:11916-11920.
[28] McAlpine JB, Banskota AH, Charan RD, Schlingmann G, et al. Biosynthesis of diazepinomicin/eco-4601, a *Micromonospora* secondary metabolite with a novel ring system. *Journal of Natural Products*. 2008;71:1585-1590.
[29] Abdelmohsen UR, Szesny M, Othman EM, Schirmeister T, et al. Antioxidant and anti-protease activities of diazepinomicin from the sponge-associated *Micromonospora* strain rv115. *Marine Drugs*. 2012;10:2208-2221.
[30] Bonfiglio G, Furneri PM. Novel streptogramin antibiotics. *Expert Opinion on Investigational Drugs*. 2001;10:185-198.
[31] Kirst HA. The spinosyn family of insecticides: Realizing the potential of natural products research. *Journal of Antibiotics*. 2010;63:101-111.
[32] Elander R. Industrial production of β-lactam antibiotics. *Applied Microbiology and Biotechnology*. 2003;61:385-392.
[33] Thykaer J, Nielsen J. Metabolic engineering of β-lactam production. *Metabolic Engineering*. 2003;5:56-69.
[34] Van Bambeke F, Glupczynski Y, Tulkens PM. Mechanisms of action. *Infectious Diseases*. 2010;7:128-135.
[35] Anderson RJ, Groundwater PW, Todd A, Worsley AJ. B-lactam antibiotics. Antibacterial agents. Chichester, UK.: John Wiley and Sons, Ltd; 2012. p. 261-303.
[36] O'Driscoll M, Greenhalgh K, Young A, Turos E, et al. Studies on the antifungal properties of n-thiolated β-lactams. *Bioorganic and Medicinal Chemistry*. 2008;16:7832-7837.
[37] Busscher GF, Rutjes F, Van Delft FL. 2-deoxystreptamine: Central scaffold of aminoglycoside antibiotics. *Chemical Reviews-Columbus*. 2005;105:775-792.
[38] Tsai A, Uemura S, Johansson M, Puglisi Elisabetta V, et al. The impact of aminoglycosides on the dynamics of translation elongation. *Cell Reports*. 2013;3:497-508.
[39] Vakulenko SB, Mobashery S. Versatility of aminoglycosides and prospects for their future. *Clinical Microbiology Reviews*. 2003;16:430-450.
[40] Hermann T. Aminoglycoside antibiotics: Old drugs and new therapeutic approaches. *Cellular and MolecularLife Sciences*. 2007;64:1841-1852.
[41] Yang G, Trylska J, Tor Y, McCammon JA. Binding of aminoglycosidic antibiotics to the oligonucleotide a-site model and 30s ribosomal subunit: Poisson-boltzmann model, thermal denaturation, and fluorescence studies. *Journal of Medicinal Chemistry*. 2006;49:5478-5490.
[42] Finch RG, Greenwood D, Whitley RJ, Norrby SR. *Antibiotic and chemotherapy e-book*. Philadelphia, American: Saunders; 2010.
[43] Jeya M, Moon H-J, Lee K-M, Kim I-W, et al. Glycopeptide antibiotics and their novel semi-synthetic derivatives. *Current Pharmaceutical Biotechnology*. 2011;12:1194-1204.
[44] Donadio S, Sosio M. Glycopeptides, antimicrobial. In: Moselio S, editor. Encyclopedia of microbiology (third edition). Oxford, UK: Academic Press; 2009. p. 455-471.
[45] Wohlleben W, Stegmann E, Süssmuth RD. Molecular genetic approaches to analyze glycopeptide biosynthesis. *Methods in Enzymology*. 2009;458:459-486.
[46] Donadio S, Sosio M. Biosynthesis of glycopeptides: Prospects for improved antibacterials. *Current Topics in Medicinal Chemistry*. 2008;8:654-666.
[47] Minotti G, Menna P, Salvatorelli E, Cairo G, et al. Anthracyclines: Molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. *Pharmacological Reviews*. 2004;56:185-229.
[48] Abdelfattah MS. Screening of terrestrial *streptomyces* leading to identification of new stereoisomeric anthracyclines. *World Journal of Microbiology and Biotechnology*. 2008;24:2619-2625.
[49] Nepomuceno JC. Antioxidants in cancer treatment. *InTech*. 2011:623-650.
[50] Kizek R, Adam V, Hrabeta J, Eckschlager T, et al. Anthracyclines and ellipticines as DNA-damaging anticancer drugs: Recent advances. *Pharmacology and Therapeutics*. 2012;133:26-39.

[51] Lynch A, Harvey J, Aylott M, Nicholas E, et al. Investigations into the concept of a threshold for topoisomerase inhibitor induced clastogenicity. *Mutagenesis*. 2003;18:345-353.
[52] Nobili S, Lippi D, Witort E, Donnini M, et al. Natural compounds for cancer treatment and prevention. *Pharmacological Research*. 2009;59:365-378.
[53] Muindi JR, Sinha BK, Gianni L, Myers CE. Hydroxyl radical production and DNA damage induced by anthracycline-iron complex. *FEBS Letters*. 1984;172:226-230.
[54] Abu-Gharbieh E, Vasina V, Poluzzi E, De Ponti F. Antibacterial macrolides: A drug class with a complex pharmacological profile. *Pharmacological Research*. 2004;50:211-222.
[55] Kanoh S, Rubin BK. Mechanisms of action and clinical application of macrolides as immunomodulatory medications. *Clinical Microbiology Reviews*. 2010;23:590-615.
[56] Zuckerman JM. Macrolides and ketolides: Azithromycin, clarithromycin, telithromycin. *Infectious Disease Clinics of North America*. 2004;18:621-638.
[57] Griffin JH, Pace JL. *Multivalent macrolide antibiotics*. Google Patents; 2007.
[58] Hansen JL, Moore PB, Steitz TA. Structures of five antibiotics bound at the peptidyl transferase center of the large ribosomal subunit. *Journal of Molecular Biology*. 2003;330:1061-1075.
[59] Gaynor M, Mankin AS. Macrolide antibiotics: Binding site, mechanism of action, resistance. *Frontiers in Medicinal Chemistry*. 2005;2:21-35.
[60] Nelson ML, Levy SB. 3.25 - *Tetracyclines and tetracycline derivatives. Comprehensive biotechnology* (second edition). Burlington, American: Academic Press; 2011. p. 269-283.
[61] Chopra I, Roberts M. Tetracycline antibiotics: Mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews*. 2001;65:232-260.
[62] Pato ML. Tetracycline inhibits propagation of deoxyribonucleic acid replication and alters membrane properties. *Antimicrobial Agents and Chemotherapy*. 1977;11:318-323.
[63] Lee M-Y, Myeong JS, Park HJ, Han K, et al. Isolation and partial characterization of a cryptic polyene gene cluster in pseudonocardia autotrophica. *Journal of Industrial Microbiology and Biotechnology*. 2006;33:84-87.
[64] Ellis D. Amphotericin b: Spectrum and resistance. *Journal of Antimicrobial Chemotherapy*. 2002;49:7-10.
[65] Paquet V, Carreira EM. Significant improvement of antifungal activity of polyene macrolides by bisalkylation of the mycosamine. *Organic Letters*. 2006;8:1807-1809.

