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Novel Bioactive Molecules from Marine Actinomycetes

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Actinomycetes are virtually unlimited sources of novel compounds with many therapeutic applications and hold a prominent position due to their diversity and proven ability to produce novel bioactive compounds. There are more than 25,000 known microbial secondary metabolites, 75% of which are produced by actinomycetes, 15% from fungi, 7% from *Bacillus* spp. and 1–2% by other bacteria. Among the actinomycetes, streptomycetes group are considered economically important because out of the approximately more than 10,000 known antibiotics, 50–55% are produced by this genus. The ecological role of actinomycetes in the marine ecosystem is largely neglected and various assumptions meant there was little incentive to isolate marine strains for search and discovery of new drugs. The search for and discovery of rare and new actinomycetes is of significant interest to drug discovery due to a growing need for the development of new and potent therapeutic agents. Modern molecular technologies are adding strength to the target-directed search for detection and isolation of bioactive actinomycetes, and continued development of improved cultivation methods and molecular technologies for accessing the marine environment promises to provide access to this significant new source of chemical diversity with novel/rare actinomycetes including new species of previously reported actinomycetes.

Key words: Novel Actinomycetes**;** Bioactive compounds**;** Marine environment; Secondary metabolites.

The sea, covering more than 70% of the surface of planet Earth, contains an exceptional biological diversity, accounting for more than 95% of the whole biosphere. Microbial diversity constitutes an infinite pool of novel chemistry, making up a valuable source for innovative biotechnology[1,2]. So far only the surface has been scratched. The recent estimates suggest that the culturability of microorganisms in marine sediments (0.25%), and especially seawater (0.001–0.10%) is considerably lower compared to soil (0.30%). A number of valuable antibiotics and metabolites have been derived from terrestrial microorganisms (99% of the known microbial compounds) although efforts in this area have diminished since the late 1980s because of the feeling that this resource has been exhaustively studied[1,3]. In this respect, researchers switched over to new environments for novel pharmaceutical compounds and for combating human pathogens

The ocean floor has been recently demonstrated as an ecosystem with many unique forms of actinomycetes. It appears that they are widely distributed throughout the ocean and found in intertidal zones, seawater, animal, plants, sponges[4, 5], and in ocean sediments. Some of the rare marine actinomycetes require seawater for their growth[6]. This kind of unique adaptation characteristic of actinomycetes [7] in the marine environment is a source of interesting research for

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new species and a promising source of pharmaceutically important compounds. The oceans are a rich and relatively available source of new microbial natural products (MNPs) in comparison with the terrestrial environment. Till date, over 23,000 bioactive compounds produced by microorganisms have been reported and more than 10,000 among these compounds were isolated from actinomycetes[8]. It is worth mentioning that of 10,000 compounds; about 80% have been obtained from *Streptomyces* which is the most productive genus in the microbial world[9]. For instance, 20 marine antitumor compounds under clinical trial, 16 are derived from microbial sources[10]. Till date, five phyla *Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes,* and *Proteobacteria* that represent 95 % of all cultivated and published species produce bioactive molecules[11]. Despite this promise, relatively little work has been done on marine actinomycetes and only a small fraction of that has been directed at examining metabolite profiles[12, 13]. Since environmental conditions of the sea are extremely different from terrestrial conditions, it is felt that marine actinomycetes may have different characteristics from terrestrial actinomycetes and therefore might produce novel bioactive compounds and new antibiotics. The research to date supports this hypothesis and it has been shown that marine actinomycetes produce novel types of new secondary metabolites[2], [14]. Many of these metabolites possess novel biological activities and have the potential to be developed as therapeutic agents. Additionally, sequencing marine actinomycete genomes may provide insights useful in the discovery of novel agents[7],[15]. This review article highlights the study of marine actinomycetes for the discovery of novel/new secondary metabolites.

Biodiversity of marine actionmycetes

Of the total sea surface, only 7–8% is coastal area and the rest is deep sea, of which 60% is covered by water more than 2000 m deep. The deep sea is a unique and extreme environment characterized by high pressure, low temperature, lack of light and variable salinity and oxygen concentration. Though the deep sea area is geographically vast, scientific knowledge and research on deep sea microbial diversity is meager. However, it has been shown to be a good source of novel microorganisms for the discovery of new antibiotics. Actinobacteria isolated from deep sea sediments in earlier studies however poorly characterized. More recently, culture independent studies have shown that indigenous marine actinomycetes certainly exist in the oceans. These include members of the genera Dietzia, Rhodococcus [16,17,18] Streptomyces[19,20], the newly described genera Salinispora[21,22,23] and Marinispor [21], [24], [25] both of which require seawater for growth and have marine chemotype signatures; and *Aeromicrobium marinum* which also has an obligate requirement for salt. Another recently characterized genus, Salinibacterium, can tolerate up to 10% NaCl but does not have a salt requirement for growth and his colleagues reported that a wide range of biologically active halophilic *Actinomycetes* was evaluated using a polyphasic approach which showed the presence of a new genus and many new species of the *Actinopolyspora, Nocardiopsis, Saccharomonospora, Streptomonospora* and *Saccharopolyspora* genera. Some of these species were found to produce unique compounds, such as salinosporamides, that are now in clinical trials as potent anticancer agents. The recently reported Verrucosispora strain AB-18-032 [26] also might qualify as an indigenous marine actinobacterium. According to Fenical and Jensen (2006)[2], representatives of at least 13 new genera of indigenous marine actinomycetes, including Salinispora and Marinispora, have been isolated and identified. Goodfellow and Fiedler (2010)[27] mention members of 50 actinomycetes genera isolated from marine sources, although only Aeromicrobium, Dietzia, Rhodococcus, Salinibacterium, Salinispora, Sciscionella and Serinicoccus genera contain or are composed of indigenous marine isolates. The recent phylogenetic diversity of marine actinobacteria isolated from the Chukchi Shelf Marine sediments in the Arctic Ocean shown that 14 different genera were isolated and identified as *Agrococcus, Arsenicicoccus, Arthrobacter, Brevibacterium, Citricoccus, Janibacter, Kocuria, Microbacterium, Microlunatus, Nocardioides, Nocardiopsis, Saccharopolyspora, Salinibacterium* and *Streptomyces*[28]. Recently, Cheng *et al*. (2015)[29] reported 23 different genera and including four putatively new species

belonging to genera *Geodermatophilus, Microlunatus, Rhodococcus* and *Actinomycetospora* in marine sponges.

The actinomycetes are active components of marine microbial communities[21] and form stable, persistent populations in various marine ecosystems. The discovery of several new marine actinomycete taxa with unique metabolic activity in their natural environments,[2] and their ability to form stable populations in different habitats and produce novel compounds with various biological activities [7,21,30,31,32, 33]) clearly illustrate that indigenous marine actinomycetes indeed exist in the oceans and are an important source of novel secondary metabolites.

Role of actinomycetes in marine environment

Actinomycetes have a profound role in the marine environment apart from antibiotic production [34, 35]. The degradation and turnover of various materials are a continuous process mediated by the action of a variety of microorganisms [21]. There is a speculation that the increase or decrease of a particular enzyme-producing microorganism may indicate the concentration of natural substrate and conditions of the environment. Metagenomic analysis of sea water column has also identified considerable number of actinomycete taxa[36]. In addition, they play a significant role in mineralization of organic matter, immobilization of mineral nutrients, fixation of nitrogen, phosphate solubilization, improvement of physical parameters and environmental protection[37].

Marine streptomycetes – a boundary microorganism

Streptomycetes are an economically important group of organisms among actinomycetes and they are the pivotal source for wide range of biologically active compounds[1]. About three quarters of all the known commercially and medicinally useful antibiotics[38] and several agriculturally important compounds were obtained from the streptomycetes. Moreover, approximately 60% of the antibiotics discovered in the year 1990 and most of the antibiotics used in agriculture are from the genus Streptomyces. Streptomycetes have been shown to have the ability to synthesize antibacterial[1,39, 40,41], insecticidal, antitumo[1,42], anti-inflammatory [43], anti-parasitic, antiviral, antifouling [44], anti-infective, nematicidal activity [45] and herbicidal and plant growth promoting compounds[45] as well as many other agents such as enzyme inhibitors and vitamins and hence, they are widely recognized as industrially important microorganisms. Further, they are well known for their capability of producing various extracellular hydrolytic enzymes including ribonucleases. These characteristics make this genus an important research subject from the academic and industrial point of view.

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[46]. 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 signaling 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[47,48]. 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[49]. Three diketopiperazine dimers including a new compound iso-naseseazine B and two known compounds naseseazine B and aspergilazine A were isolated from marine actinomycets [11]. Recently, Wu *et al*. (2016)[50] identified the secondary metabolites like isocoumarins, undecylprodiginine, streptorubin B, 1H-pyrrole-2-carboxamide, acetyltryptamine and fervenulin were produced in *Streptomyces* sp. MBT76. Another two prominent examples of studies into the biosynthesis of secondary metabolites from marine actinomycetes

include those for cytotoxic compounds thiocoraline and BE-14106. Lombo *et al*. (2006)[51] have cloned and analyzed thiocoraline biosynthesis gene cluster from marine *Micromonospora* isolate.

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[52]. 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[53]. 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[54]. Genome mining for new candidate secondary metabolic pathways based on clustering and coexpression has proved to be a highly successful approach in microbes [55]. 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[56]. Actinomycetes continue to be a productive and successful focus for natural products research, with many novel compounds been of eminent pharmacological valuable.

Antibiotics compound 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.

Aminoglycosides

Streptomycin (Fig.1) is an antibiotic drug, the first of a class of drugs called aminoglycosides to de discovered, and it was the first antibiotic remedy of tuberculosis. It is derived from the actinobacterium *Streptomyces griseus*. Streptomycin is a bactericidal antibiotic. Generally, aminoglycosides are protein synthesis inhibitors. Tobramycin, kanamycin, paromomycin and spectinomycin (Fig. 1) were an aminoglycoside antibiotic derived from *Streptomyces tenebrarius, Streptomyces kanamyceticus*, *Streptomyces krestomuceticus* and *Streptomyces spectabilis* and used to treat various types of bacterial infections. Neomycin (Fig. 1) is an aminoglycoside antibiotic derived from Streptomyces fradiae and it was in many topical medications such as creams, ointments and eyedrops.

β-lactams, Glycopeptides, polyenes and actionomycins

Cephamycins (Cephalosporins) (Fig. 2)

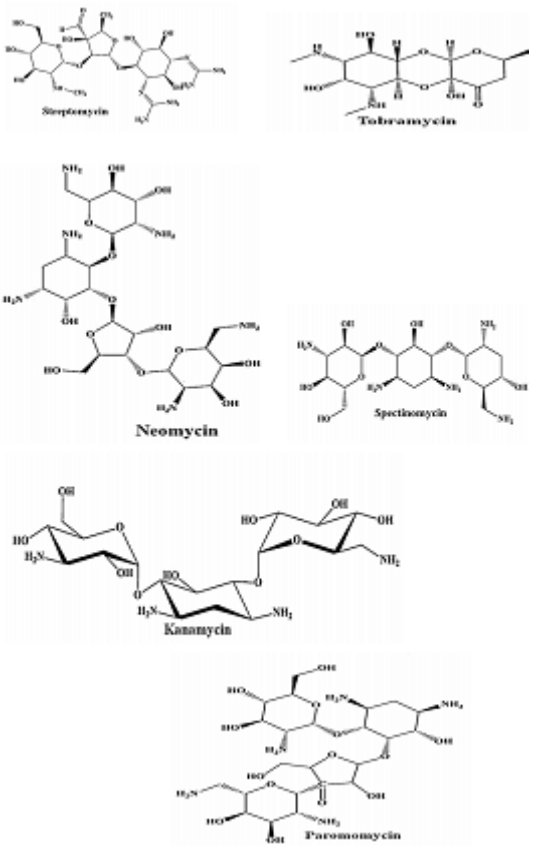


Fig. 1. Chemical structure of aminoglycoside

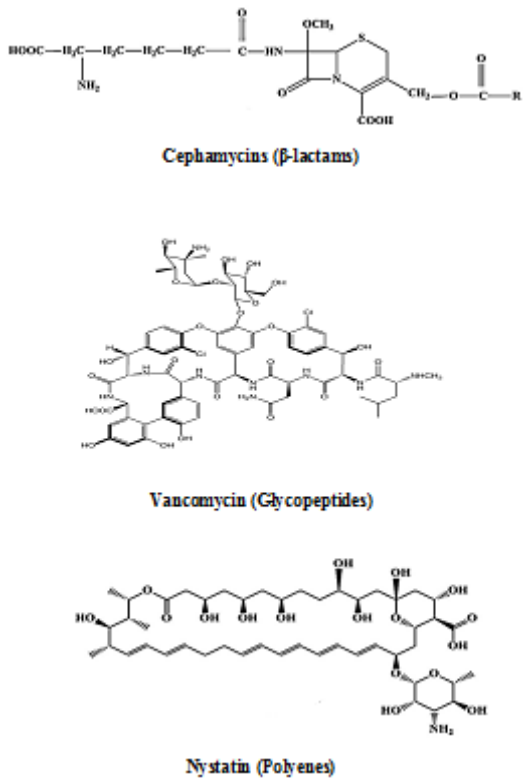


Fig. 2. Chemical structure of β-lactams, Glycopeptides, polyenes and actionomycins Peptides

are a group of β-lactam antibiotics from *Streptomyces* indicated for use against many bacterial infections. Vancomycin (Fig. 2) is a glycopeptides antibiotic used in the prophylaxis and treatment of infection caused by Gram-positive bacteria. It is a naturally occurring antibiotic made by the soil actinobacterium Amycolatopsis orientalis. Nystatin (Fungicidin) (Fig. 2) is a polyene antifungal medication to which many molds and yeast infections are sensitive, including Candida. These polyene antimycotics are typically obtained from some species of S*treptomyces*. Actinomycin (Fig. 2) is the most significant

Peptides

Peptides are short chains of amino acids. Most of the actinobacterial peptides are cyclic and contain further rare structural elements such as chromophores or uncommon amino acids. Peptides include mechercharmycins which are the new cytotoxic substance obtained from marine-derived *Thermoactinomyces* sp. YM3-251. Cyclic nature of mechercharmycin A (Fig. 3) exhibited relatively strong antitumor activity and the related compound, mechercharmycin B (Fig. 3) exhibited no such activity[57]. Thiocoraline (Fig. 3) is a novel cyclic thiodepsipeptide isolated from *Micromonospora* sp. L-13-ACM2-092 and it showed a potent antitumor activity against P388, A549, and MEL288. There was also a strong antimicrobial activity against Gram-positive microorganisms[58],[59]. Cyclomarin A (Fig. 3) is a new cyclic heptapeptide produced by a *Streptomyces* sp. It showed significant antiinflammatory activity in both in vivo and in vitro assays[43]. Piperazimycins (Fig. 3) are cytotoxic hexadepsipeptides obtained from the fermentation broth of a *Streptomyces* sp. strain CNQ-593, isolated from the marine sediments at a depth of approximately 20 m near the island of Guam. Piperazimycin A exhibited potent in vitro cytotoxic activity against the human colon

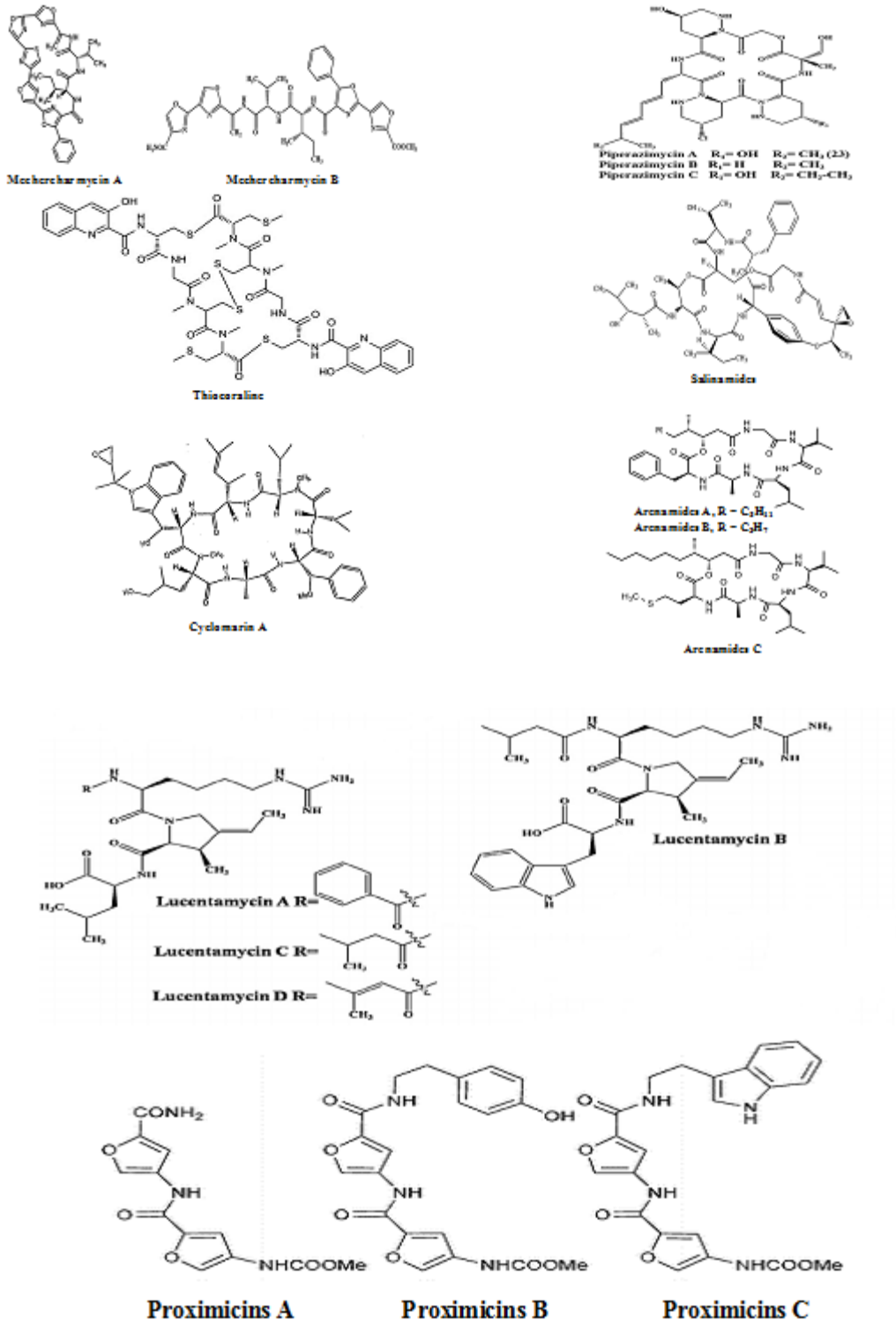


Fig. 3. Chemical structure of Peptides

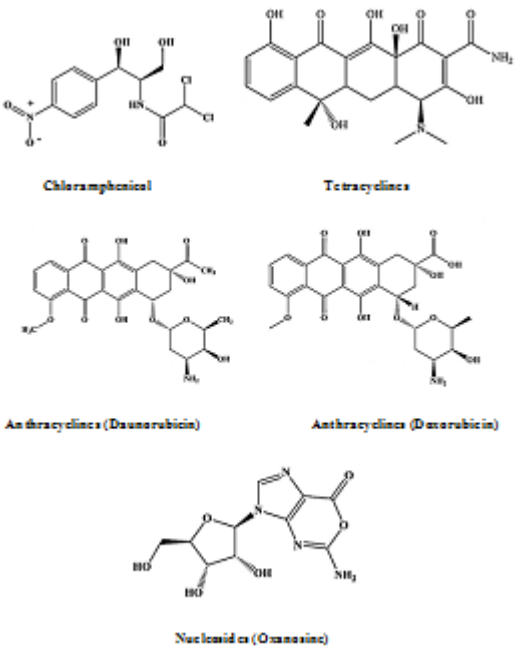


Fig. 4. Chemical structure of Chloramphenicol, tetracyclines, anthracyclines and nucleosides

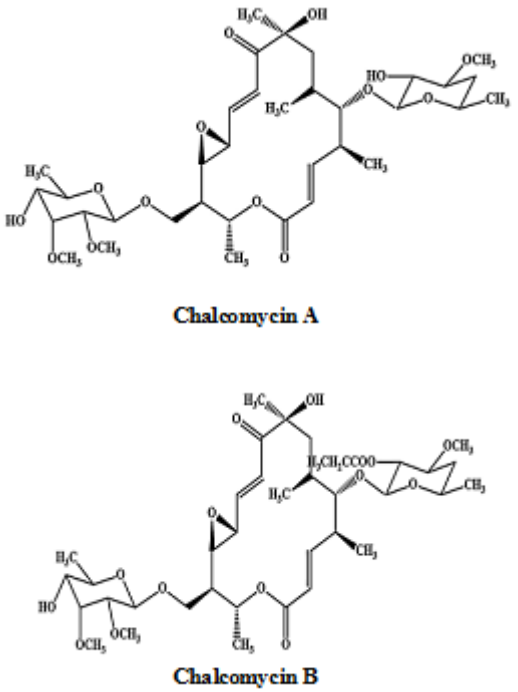


Fig. 5. Chemical structure of Macrolides

carcinoma HCT-116 cell line[60].

Salinamides A and B (Fig. 3) are bicyclic depsipeptides, produced by *Streptomyces* sp. CNB-091, isolated from the jelly fish *Cassiopeia xamachana*. These metabolites are valuable as antibiotic and anti-inflammatory agents [61]. Arenamides A–C (Fig. 3) are three new cyclohexadepsipeptides isolated from the fermentation broth of a marine actinobacterial strain identified as *Salinipora arenicola* CNT-088 which was obtained from the marine sediment sample collected at a depth of 20 m off the Great Astrolab Reef, in the Kandavu Island chain, Fiji, in 2006. Arenamides A and B showed weak in vitro cytotoxicity against human colon carcinoma HCT-116 with IC50 values of 13.2 and 19.2 g/ml, respectively. The effect of arenamides A and B on NFêB activity was studied with stably transfected 293/NFêB Luc human embryonic kidney cells induced by treatment with tumor necrosis factor (TNF). Lucentamycins A–D (Fig. 3) are four new 3-methyl- 4-ethylideneproline-containing peptides isolated from *Nocardiopsis lucentensis* strain CNR-712, obtained from the sediments of a shallow saline pond from the island of Little San Salvador, the Bahamas. Lucentamycins A and B exhibited significant in vitro cytotoxicity against human colon carcinoma cell line HCT-116 with IC50 values of 0.20 and 11 M, respectively. However, lucentamycins C and D were not cytotoxic in the same assay suggesting that the presence of an aromatic ring is essential for the biological activity of this class of compounds[62]. Proximicins are novel aminofuran antibiotics produced by Verrucosispora strain MG-37 isolated from the sediments collected in the Raune Fjord, Norway at a depth of 250 m and the Sea of Japan at a depth of 289 m[26,63]. The characteristic structural element of proximicins is 4-amino-furan-2-carboxylic acid, a hitherto unknown ã-amino acid. Fig. 3 has shown the structure of Proximicins A (a), B (b) and C (c). It has an inhibitory effect against the human gastric adenocarcinoma AGS and hepatocellular carcinoma Hep G2[64].

Chloramphenicol, tetracyclines, anthracyclines and nucleosides

Chloramphenicol (Fig. 4) is an antibiotic derived from *Streptomyces venezuelae* and it was in the treatment of typhoid[65]. Tetracycline (Fig. 4) is a borad-specturm antibiotic produced by the

Streptomyces sp. indicated for use against many bacterial infections[66]. Anthrocyclines are a class drugs used in cancer chemotherapy derived from *Streptomyces peucetius*. The first anthracycline discovered was daunorubicin and doxorubicin (Fig. 4) which is produced naturally by *S. peucetius*, a species of actinobacteria[67]. Oxanosine (Fig. 4) was isolated as a novel nucleoside antibiotic from *Streptomyces capreolus* MG265-CF3[68].

Macrolides

Macrolides are a cluster of drugs whose activity stems from the presence of a macrolide ring, a large macrocyclic lactone ring to which one or more deoxy sugars may be attached. Generally, macrolides are protein synthesis inhibitors. Chalcomycin A (Fig. 5) is a macrolide antibiotic obtained from the crude extract of the marine *Streptomyces* sp. M491 from the Qingdao coast (China). It exhibited activity against *E. coli*, *B. subtilis*, and *S. aureus* and a weak inhibition of S. *viridochromogenes* in biological screening[69]. Chalcomycin B (Fig. 5) is a new macrolide antibiotic obtained from the culture broth of a marine *Streptomyces* sp. B7064, isolated from the mangrove sediments near Pohoiki, Hawaii (Pacific Ocean)[70].

Antimalarial compound produced by actinomycetes

Malaria is a highly infectious disease caused by a protozoan parasite of the genus *Plasmodium*. The transmission of this parasite is by the bite of infectious female Anopheles sp mosquitoes. Five species of Plasmodium are associated with malarial fever viz, P. *falciparum, P. vivax, P. Malariae, P. ovale* and *P. knowlesi*. Among which, *P. falciparum* is extremely virulent. While, *P. vivax* is relatively less virulent and is more common in all over the world and remain three species are linked to the slight outbreak in several parts of the world. For the control of more virulent malarial parasite *Plasmodium falciparum*, three approaches were considered. It includes the step up of effective vaccines, vector control and development of new drugs. Due to the exhibition of multiple antigenicity, the development of a vaccine becomes difficult. The vector control shows limited success. On the other hand, malarial parasites show increasing resistance to the existing drug hence, there is an urgent need for new antimalarial agents[71,72]. There is a need of new drug that ideally directed against new targets such as heme and malarial proteases. However, the isolation of novel enzyme inhibitor from terrestrial sources is uncommon hence marine actinobacteria will provide new potential inhibitors.

Trioxacarcins are the complex compounds which showed higher anti-malarial activity against the malarial pathogens and some of them possess high antitumor and antibacterial activities.

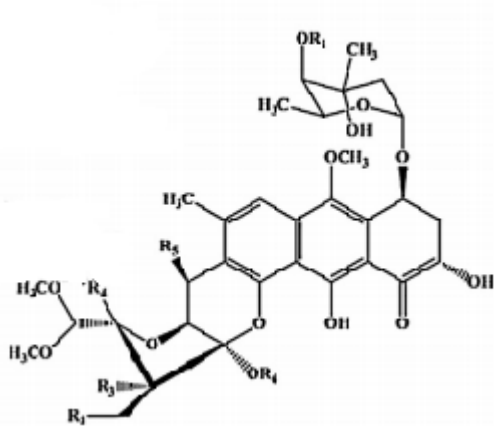


Fig. 6. Chemical structure of Trioxacarcin

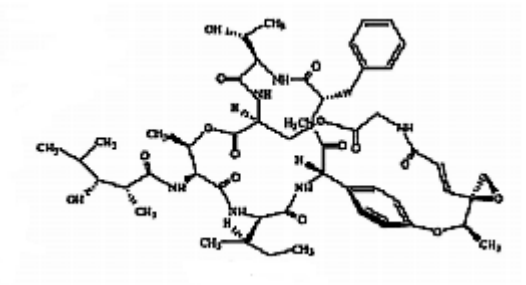


Fig. 7. Chemical structure of Salinamides

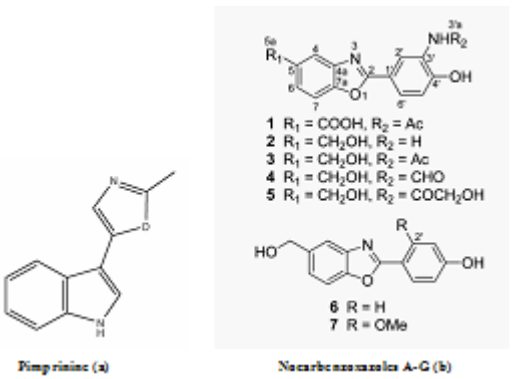
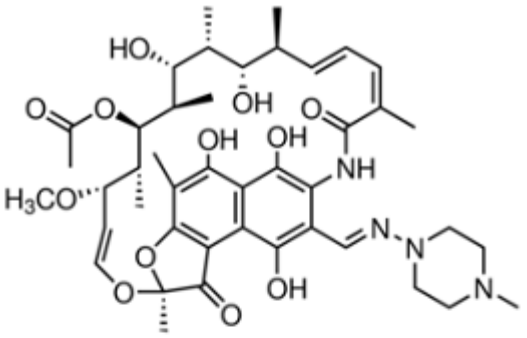


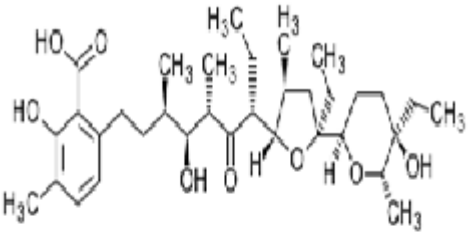
Fig. 8. Chemical structure of Anti-tumor compounds

Trioxacarcin A, B and C (Fig. 6) obtained from *Streptomyces ochraceus* and *Streptomyces bottropensis*[73]. Some of these compounds possess extremely high antiplasmodial activity, which is comparable to that shown by artemisinin, the most active compound against the pathogen of malaria.

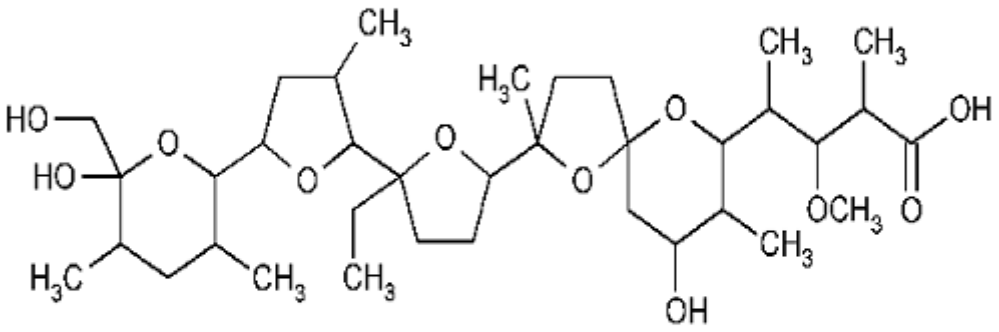
New secondary metabolites salinos poramide A (Fig. 7) screened from marine actinomycetes tropica, is a highly potent inhibitor of the human malaria parasite *in vitro* and *in vivo*. Moderately conserved sequences of the proteasome subunits across species and



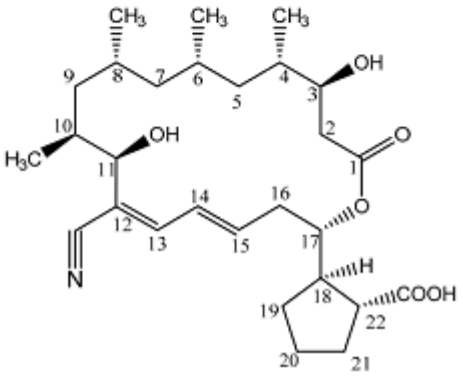
Rifampicin (a)



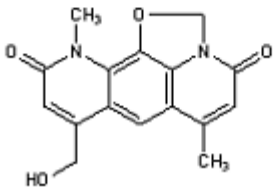
Lasalocid (b)



Monensin (c)



Trepenomycin (d)



Nybomycin (e)

Fig. 9. Chemical strucutre of Antitubercular compounds

exponential growth of the parasite in red blood cells, suggests proteasome as the probable target of salinosporamide A in the malaria parasite. The inhibitory effect of salinosporamide with an IC50 of 11.4 nM is as competent as the majority of conventional antimalarials such as artemisinin or chloroquine. The pure compound, salinosporamide A, was tested for its inhibitory effect against parasite development *in vitro* (*P. falciparum*) and *in vivo* (*P.yoelii*). The biochemical and structural-based analyses are reliable with the parasite 20S proteasome being the molecular target. The divergence at the structural level facilitates the discovery of an increased specificity of Plasmodium proteasome inhibitors[30].

Today, there is an urgent need for discovering the new small and inexpensive antimalarials molecules. Using a [3H] hypoxanthine incorporation assay on the parasite's erythrocytic stages, it was determined that secondary metabolites produced by marine actinomycetes have significant antimalarial activities. These findings displays that the natural products are the most important sources of medicines against the parasite. The crude extract was obtained from marine actinomycetes isolated from the mangrove and sea grass sediments of Dar es Salaam, Tanzania. The hypoxanthine incorporation assay was used to determine parasite inhibition profiles and 50% inhibitory concentration values of the Actinomycetes crude extracts. The three crude extracts showed strong growth inhibition with an IC50 in the low microgram range (0.44 – 7.98 ìg/ml)[74].

Anti-tumor compounds produced by actinomycetes

Although, recent advances have been done in the field of the molecular biology of cancer stimulation and progression in its different forms, it is still one of the most threatening illnesses which affect human health and quality of life[75]. One of foremost problem in treatment of various infectious diseases and cancer is the multidrug resistance. There is a need to develop a drug with high great novelty, potency and less toxicity. Marine microbes especially *Streptomyces* sp., is the major source of the antitumor antibiotics. When these antitumor antibiotics interact with DNA it results to cause cell death[76]. Anthracyclines are a special class of antitumor antibiotics which act via topoisomerase II inhibition[77]. An extracellular alkaloid, Pimprinine has (Fig. 8a) been isolated from the culture filtrate of *Streptomyces* CDRIL-312[78]. The novel aporphine alkaloid SSV isolated from bioactive *streptomyces* sp. KS1908, is an antitumor antibiotic obtained from fraction of fermented broth and chemically characterized with advanced spectroscopic data. Hence, aporphine alkaloid SSV has been act as a promising agent for treatment of cancer and

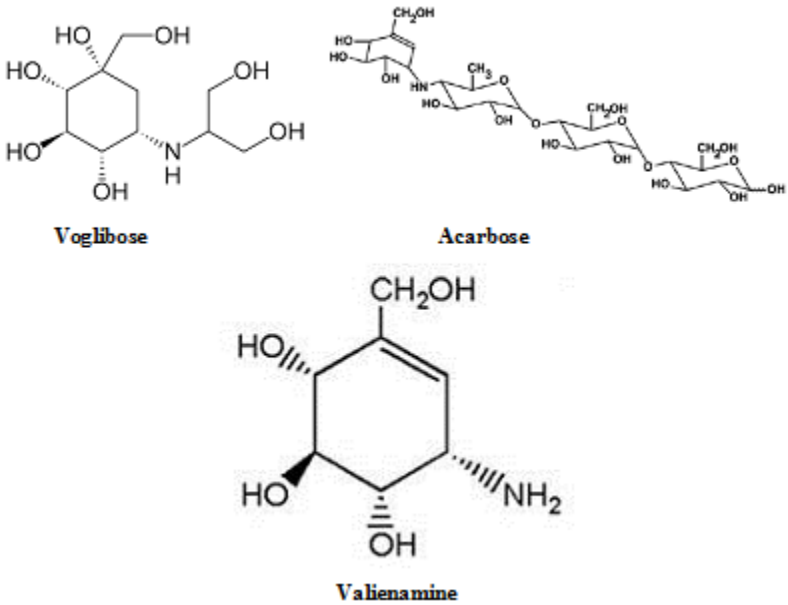


Fig. 10. Chemical structure of Anti-diabetic compound

microbial infections[79]. Seven new benzoxazole derivatives, nocarbenzoxazoles A"G (Fig. 8b) were isolated from the halophilic strain *Nocardiopsis lucentensis* DSM 44048, act as an antitumor activity and structure was elucidated by using NMR spectrometric analysis[5].

Antitubercular compounds produced by actinomycetes

Tuberculosis (TB) is a highly infectious disease caused by *Mycobacterium tuberculosis*, is one among the leading cause of infectious disease worldwide. One of the world population suffered with *M. tuberculosis* and hence at a risk of active TB[80]. In the late of 1940s and 1950s, a number of effective antitubercular agents were discovered, with rifampicin (Fig. 9a) getting introduced in 1960s [81]. In 1940s, Streptomycin was the first drug to be introduced for the treatment of TB but immediately after its introduction many of the patients started showing resistance against this antibiotic[82]. Multidrug resistance (combined isoniazid and rifampicin) strongly impacts on control program of TB. However, there is an urgent to discover novel antibiotics against drug resistance *M. tuberculosis*.

The two natural antibiotics, Lasalocid and Monensin (Fig. 9b and 9c) isolated mainly from fungal mycelium of *Streptomyces cinnamonensis* (Actinomycetes). These antibiotics belong to the family of the polycyclic carboxylic polyethers [83]. Monensin and lasalocid and their metal complexes with Tl (I), La (III) and Gd (III) are active against *Mycobacterium tuberculosis*. These ionophores could be considered as potential antitubercular agents for the future discovery of new drug design[84]. Recently, *Streptomyces mutabilis* isolated from soil samples in Saudi Arabia produced an anti-tuberculous polyketide macrolide identified as treponemycin (Fig. 9d)[85]. Nybomycin (Fig. 9e) isolated from the culture broth of a marine derived *Streptomyces* sp. showed potent anti-microbial activity against *Mycobacterium* sp. in both actively growing aerobic conditions and dormancy induced hypoxic conditions. In addition, compound was also effective to pathogenic strains of *M. tuberculosis* including clinically isolated strains[86].

Plant endophytes represent the rare and previously undiscovered microbial taxa to reduce the rediscovery of known compounds. 50 strains and 300 crude extracts in total were isolated from traditional Chinese medicines (TCMs) for growth inhibitory activity against Bacillus Calmette- Guérin (BCG)[87], an attenuated strain of the bovine tuberculosis bacillus *Mycobacterium bovis*. The crude extract of *Streptomyces* sp. strain Y3111 associated with the stems of *Heracleum souliei*, showed good anti-BCG activity with an MIC value of 12.5µg/mL. The Bioassay-guided fractionations

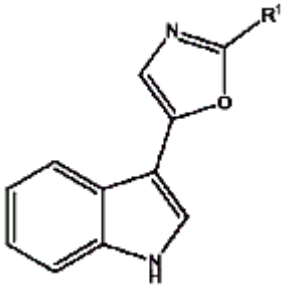


Fig. 11. Chemical structure of Pimprinethine

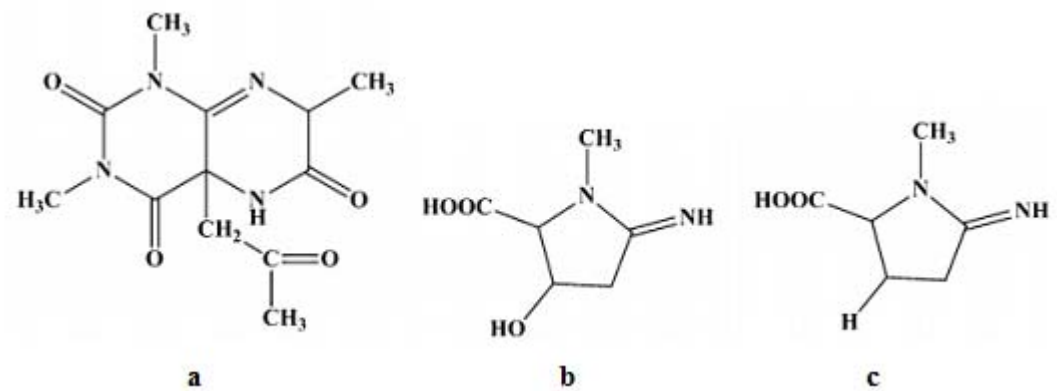


Fig. 12. Chemical structure of Pyrizinostatin (a), Pyrostatin A (b) and Pyrostatin B (c)

yield the structurally diverse heraclemycins A–D. The structures were determined by different spectroscopic techniques including HRMSESI, 1D NMR, and 2D NMR. Heraclemycin C showed strong anti-BCG activity with a MIC value of 6.25ìg/mL. This inferred that the endophytic microbes associated with TCMs can facilitate the discovery of new natural products and potentially new and novel molecular scaffolds for drug discovery[88]. Nybomycin isolated from the culture broth of a marine derived *Streptomyces* sp. showed potent anti-microbial activity against Mycobacterium sp. in both actively growing aerobic conditions and dormancy induced hypoxic conditions. In addition, compound 1 was also effective to pathogenic strains of *M. tuberculosis* including clinically isolated strains.

Anti-diabetic compound produced by actinomycetes

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia, negative nitrogen balance sometime ketonemia and glycosuria. Resulting either from inadequate secretion of insulin, an inadequate response of target cells to insulin, or combination of these factors. Insulin deficiency is due to functional disorder of the pancreas.

Diabetes mellitus is the highest cause of death among other chronic diseases. More than 95% of diabetes is type 2 diabetes or often called non-insulin dependent diabetes [89]. This cannot be cured, but can be controlled. Hence major efforts have been directed towards the development of oral hypoglycaemic drugs, to identify compounds able to enhance insulin action in target tissues. Attention has been focused on searching from terrestrial microorganism to look for new sources of drug and many new families of antibiotics are found from these microorganism.

For the treatment of type-2 diabetes; Voglibose, Acarbose, valienamine, adiposin-1, and trestatin-B were reported from Streptomyces hygroscopicus-limoneus [90],[91], S. calvus[92],[93], and S. dimorphogenes[84] respectively. Voglibose (Fig. 10) is an alpha-glucosidase inhibitor that lowers the post prandial blood glucose levels in people with diabetes mellitus. It is produced and marketed in India by trade name Volix® and Vocarb® as[90]. Acarbose (Fig. 10) is an oral alpha-glucosidase and alpha-amylase inhibitor, first launched by Bayer in Switzerland in 1989 for the oral treatment of type-2 diabetes mellitus[95]. Valielamine (Fig. 10), a precursor of voglibose and a new aminocyclitol, isolated from the fermentation broth of

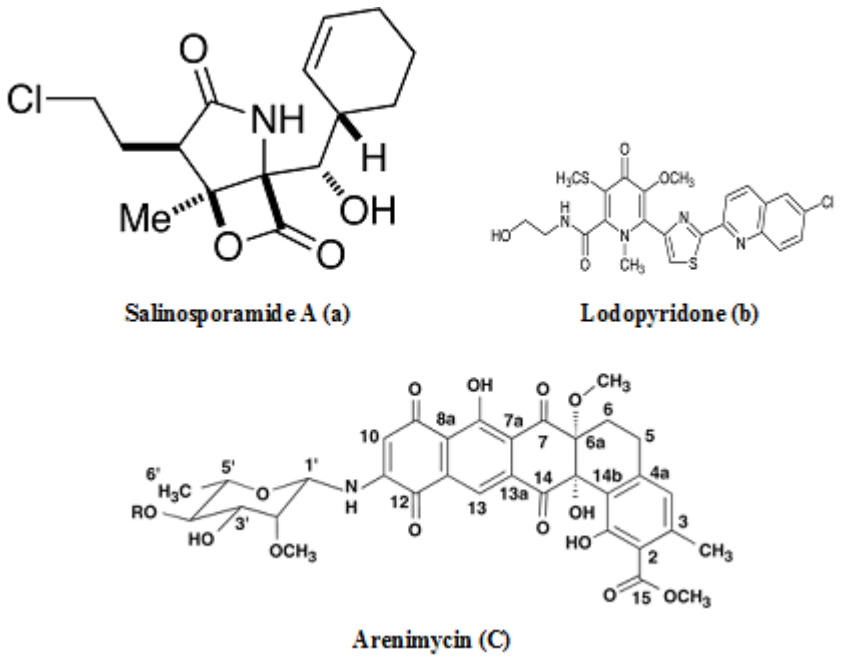


Fig. 13. Chemical structure of Salinosporamide A (a); Lodopyridone (b); Arenimycin (C)

Streptomyces hygroscopicus subspecies limoneus. It has more potent a-glucosidase inhibitory activity against porcine intestinal sucrase, maltase and isomaltase than valienamine, validamine and hydroxylvalidamine which were reported as building blocks of validamycins and microbial oligosaccharide a-glucosidase inhibitors [96].

Insecticidal activity of actinomycetes

As the environmental contamination increases by toxic chemicals, for this alternative approaches for controlling pest populations have become research priorities. For limiting the destructive impacts of pest populations, ecological or biological control methods has evolved [97],[98].

Actinomycetes play a significant role in the biological control against insect together with the house fly *Musca domestica*[99], *Culex quinquefasciatus*[100], cotton leaf worm *spodopetra liltoralis*[101], *Drosophila melanogaster*[102], *Helicoverpa armigera*[103], *Anopheles mosquito* larvae[104] and *Culex pippins*[105].

The biological activity of secondary metabolites of some actinomycetes isolates had been investigated against the last instar larvae of the cotton leaf worm *Spodoptera littoralis* through the food plant (Castor leaves). *Streptomyces* and *Streptoverticillum* were the most potent actinomycetes, which cause larval and pupal mortality[106]. The biological activities of actinomycetes secondary metabolites against some phytopathogenic fungi as well as cotton leaf worm (*S. littoralis*) were investigated. They showed that the pellets of some *Streptomyces* isolates were more active against cotton leaf worm than culture filtrates. Generally, isolates S05, S08, S10 and S15 showed 80, 100, 70 and 80% mortality against cotton leaf worm, respectively. The result demonstrated the ability to use such *Streptomyces* isolates as effective biopesticide agents[107].The purified compound, aminoglycosidic antibiotic produced from *Streptomyces bikiniensis* is also effective against 2nd instar larvae of cotton leaf worm *Spodoptera littoralis*[108].

Table 1. Secondary metabolites isolated from marine actinomycetes from 2010 to 2015

Organism	Compound	Activity	Reference
Dermacoccus sp.	Dermacozines	Cytotoxic, radicalscavenging	Abdel-Mageed *et al.* 2010[135]
Nocardiopsis sp.	TP-1161	Antibacterial	Engelhardt *et al.* 2010[136]
Streptomyces sp.	2-Allyloxyphenol	Antimicrobial, antioxidant	Arumugam *et al.*2010[137]
Streptomyces sp.	ML-449	Cytotoxic	Jørgensen *et al.*2010[138]
Micromonospora haikouensi	menaquinones	Antitumor	Xie *et al.* 2012[139]

Antivirial activity of actinomycetes

Marine antiviral agents (MAVAs) represent a significantly unique natural marine resource whose multi-potential uses include the following applications: The biological control of human enteropathogenic virus contamination and disease transmission in sewage-polluted waters. This application would be particularly important to communities that utilize their coastal waters for recreational activities and for food industries (e.g. fish, shellfish), as well as to those regions of the country, such as Hawaii, where the loss of these marine resources would have a devastating effect on the lifestyle and economy of the people and the chemotherapy of viral diseases of humans and lower animals[109]. Wei *et al.* (2014)[110] isolated a compound Pimprinethine (Fig. 11) and its derivatives from *Streptomyces* sp, exhibited inhibitory activity against EV71 and ADV-7, slightly activity against CVB3, HSV-1 and H1N1, with a few exceptions, in vitro. These compounds mainly act at the early stage of the EV71 replication period.

Enzyme inhibitors from actinomycetes

Enzyme inhibitors have been obtained increasing attention as useful tools, not only for the study of enzyme structures and reaction mechanisms but also for potential utilization in pharmacology[111]. Imada (2005)[112] reported different types of enzyme inhibitors viz. β-glucosidase, N-acetyl-β-D-glucosaminidase, pyroglutamyl peptidase, á-amylase inhibitors from marine actinobacteria and act as the potential source for

production of enzyme inhibitors. Pyrizinostatin (Fig. 12) is an inhibitor of pyroglutamyl peptidase, isolated from culture of *Streptomyces* sp. SA-2289[113]. Pyrostatin A and B are inhibitors of N-acetyl-β-glucosaminidase, produced by *Streptomyces* sp. SA-3501[111, 112]. Raja *et al.* (2010)[114] isolated 17 marine actinobacteria strains from the rhizosphere sediments of mangroves and reported the amylase inhibitor. Ganesan *et al.* (2011)[115] studied three marine actinobacteria from Parangipettai coast, east coast of India and reported the β-glucosidases inhibitory activity

Vaccines from actinomycetes

A common problem associated with infectious diseases is chronification of the process. This might be due to an inadequate immune response of the patient or to decreased sensitivity to anti-infective drugs used to treat the disease causing micro-organism. Chronic infections can lead to severe tissue damage and chronically infected individuals can act as a permanent source for the transmission of clinically important bacteria or viruses into the wider population. In addition, the treatment of chronic or recurrent infection is often prolonged and complicated. An early attempt at treating chronic, recurrent and acute infections was the use of the so-called autovaccine[116]. Autovaccination was regularly performed in human medicine at the beginning of the 20th century. However, since that time, with the exception of some eastern European countries, the use of autovaccination therapy steadily decreased. Today, autogenous vaccines or autovaccines are commonly used in veterinary medicine to treat chronic infectious diseases[117]. Jost *et al.* (1999) [118]report that insertional inactivation of the *A. pyogenes plo* gene results in decreased virulence of the *plo* mutant and that PLO, like other TACYs, is cytotoxic for phagocytic cells. In addition, we provide evidence that recombinant His-tagged PLO (His-PLO) is efficacious as a vaccine in mice.

Strain improvement of actinomycetes

Methods for efficient chemical mutagenesis and recombination by protoplast fusion have been available for strain improvement in actinomycetes for over a quarter of acentury[119], [120]. More recently, mutagenesis and protoplast fusion have been combined for whole genome shuffling to improve the production of antibiotics[4],[44] ,[121]. In addition, a number of molecular engineering strategies, often focused on optimizing precursor levels or expression of rate-limiting enzymes involved in secondary metabolite biosynthesis, have been summarized in other reviews[31,122,123, 124]. The mutagenesis, recombination, and molecular engineering approaches continue to be very useful tools for industrial-scale strain improvement. Chemical mutagenesis [e.g., by N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), ethyl methanesulfonate (EMS), or nitrous acid (NA)] and physical mutagenesis [e.g., by ultraviolet (UV) light or X-rays)] remain the single most robust methods to generate very high-producing strains for SM production over campaigns spanning many years[125]. Li *et al.* (2013)[126] reported that the effect of pleuromutilin resistance on antibiotic production in *Streptomyces*. The results showed that Pler mutants could increase the production of daptomycin by approximately 30%. For genomics applications, the cloning and heterologous expression of silent or poorly expressed secondary metabolite gene clusters provides another approach to produce sufficient yields for isolation and characterization of novel compounds. This approach may be particularly useful for the expression of cryptic pathways from rare actinomycetes that grow slowly or are not amenable to industrial-scale fermentation for other reasons. There are a number of laboratory strains that have been used to express heterologous secondary metabolite gene clusters[127, 128].

Novel/New metabolites from marine actinomycetes

Although more than 30,000 diseases have been clinically described, less than one third of these can be treated symptomatically and fewer can be cured. New therapeutic agents are urgently needed to fulfill the medical needs that are currently unmet. Natural products once played a major role in drug discovery. Although the exploitation of marine actinomycetes as a source for discovery of novel secondary metabolites is at an early stage, numerous novel metabolites have been isolated in the past few years. **Table 1** shows some examples of new secondary metabolites isolated from marine actinomycetes from 2010 to 2015. This is by no means an exhaustive search of all novel secondary metabolites produced by marine actinomycetes during this 5-year period, nevertheless this list is

impressive and illustrate the many different diverse structures with biological activities reported. Among them, a few compounds such as staurosporinone, salinosporamide A, lodopyridone, arenimycin, marinomycins and proximicins from Table 1 are of particular interest due to their rarity and potent and diverse bioactivity. Salinosporamide A (Fig. 13a) is a novel rare bicyclic beta-lactone gamma-lactam isolated from an obligate marine actinomycete, *Salinispora tropica*[7]. Salinosporamide A is an orally active proteasome inhibitor that induces apoptosis in multiple myeloma cells with mechanisms distinct from the commercial proteasome inhibitor anticancer drug Bortezomib. It is being developed by Nereus Pharmaceuticals, Inc. (as NPI-0052) and was scheduled to enter clinical studies for treatment of cancer in humans in 2006. NPI-0052 is currently being evaluated in multiple phase I trials for solid tumors, lymphoma and multiple myeloma.

Lodopyridone (Fig. 13b) is a unique alkaloid produced by a marine *Saccharomonospora* sp. isolated from marine sediments collected at the mouth of the La Jolla Submarine Canyon[129]. Lodopyridone possess activity against the human colon adenocarcinoma cell line HCT-116 with an IC50 of 3.6 µM. Arenimycin (Fig. 13c) is a new antibiotic belonging to the benzo (â) naphthacene quinone class produced by the obligate marine actinomycete, *Salinispora arenicola*[32]. This new structural derivative is the first report of this class of antibiotics from this strain *S. arenicola*. Arenimycin has effective antibacterial activity against rifampin- and methicillin-resistant *Staphylococcus aureus* and it exhibits potent antimicrobial activities against drug resistant *Staphylococci* and other Gram-positive human pathogens. In addition, the *Salinispora tropica* strain also produces four unprecedented compounds, Sporolide A, Arenicolide A, Cyanosporaside A and Salinispyrone A[7]. These highlighted structures demonstrate the tremendous potential of marine actinomycetes for the production of novel secondary metabolites. Lipoxazolidinones A–C is a novel 2-alkylidene-5-alkyl-4-oxazolidinones isolated from novel and rare genus *Marinispora*. The biological activity of lipoxazolidinones exhibited broad spectrum antimicrobial activity similar to that of the commercial antibiotic linezolid (Zyvox), a 2-oxazolidinone[130]. Lynamicins A–E is a chlorinated bisindole pyrroles isolated from the rare *Actinomycete Marinispora* sp[131].

Wang *et al*. (2013) [132]reported an array of new and novel abyssomicins, a new class of unique polycyclic natural products with potent antibacterial, antitubercular, antitumor and anti-Bacille Calmette Guerin activity by *Verrucosispora* spp. Gifhornenolones A and B are new terpenoids isolated from the marine ascidian-associated *Verrucosispora gifhornensis*. The biological activity of gifhornenolone A showed potent inhibitory activity to the androgen receptor[133]. Thiochondrillines analogs of thiocoraline, are potent cytotoxic thiodepsipeptides isolated from the spongeassociated *Verrucosispora* sp[134].

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CONCLUSION

As the scope of naturally found bioactive compounds increases day by day, search for novel active biomolecules increases. From the last many decades research on bioactive molecules produced from marine microorganism has geared up. Among all actinomycetes has proved to be the best. These are diverse in nature having various chemical entities responsible for the activity against many other disease causing microorganisms. Hence these bioactive compounds are considered to be as potent therapeutic agents. However, actual potential of actinomycetes is yet to be discovered. In future we will discover the new activities of microbial metabolites and expand their practical utilization.

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