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REVIEW ARTICLE

# Cyanobacteria: an emerging source for drug discovery

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**The c group of Gram-negative gliding bacteria, has a long history of cosmopolitan occurrence. It has great biodiversity despite the absence of sexual reproduction. This wide biodiversity may be reflected in the wide spectrum of its secondary metabolites. These cyanobacterial secondary metabolites are biosynthesized by a variety of routes, notably by non-ribosomal peptide synthetase or polyketide synthetase systems, and show a wide range of biological activities including anticancer, antibacterial, antiviral and protease inhibition activities. This high degree of chemical diversity in cyanobacterial secondary metabolites may thus constitute a prolific source of new entities leading to the development of new pharmaceuticals.**
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## INTRODUCTION

The main focus in recent decades for pharmaceutical discovery from natural products has been on microbial sources (bacterial and fungal), dating back to the discovery of penicillin from the mould fungus *Penicillium notatum* in the first half of the twentieth century.[1] The emphasis on terrestrial microbes has more recently been augmented by the inclusion of microbes from marine sources. The majority of new chemical entities introduced as drugs (60 and 75% in areas of cancer and infectious disease, respectively) in market during the period of 1981–2002 are of natural origin.[2,3]

In recent years, the pharmaceutical industry has invested in high-throughput screening systems, genomics and bioinformatic tools, rational design and combinatorial chemistry for the discovery of new bioactive compounds. Despite of these technological advances, the number of new entities reaching the market has declined from 53 to only 26 within a time span of 9 years (1996–2005). Currently, the pharmaceutical industry has to spend around 500 million to 2000 million dollars, depending on the therapy or the developing firm, to bring a new drug (a drug with a new chemical entity) to market.[4] As a result, there is a scarcity of new therapeutic drugs reaching the market. On the other hand, the prevalence of diseases such as cancer, human immunodeficiency virus (HIV)–acquired immune deficiency syndrome, hematological and autoimmune disorders is increasing rapidly.

Currently available drugs are effective against only one-third of the diseases as a result of increased antibiotic resistance in pathogens. Thus, identification of new biologically active compounds is urgently required for development of new drugs. To fulfill the demand for new therapeutic drugs and to decrease the average costs involved in development, scientist should consider screening organisms from overlooked microbial sources, such as proteobacteria, bacterioidetes and cyanobacteria. The medicinal qualities of cyanobacteria were first recognized in 1500 BC when *Nostoc* sp. were used to treat gout, fistula and several forms of cancer[5] but the most extensive modern work in this field was started in the 1990s by RE Moore and WH Gerwick.

## CYANOBACTERIA

Cyanobacteria, a group of Gram-negative photoautotrophic prokaryotes, are capable of performing oxygenic photosynthesis. This unique class of microorganisms has many names, like cyanoprokaryotes, cyanophytes and blue–green bacteria. These names are derived because of the presence of a blue–green colored pigment, c-phycocyanin (C-PC), which is a pigment used for photosynthesis. These bacteria have a fossil record from 3.3 to 3.5 billion years,[6] and are still among the most successful organisms on earth. They have a typical prokaryotic cell organization but like eukaryotes have an elaborate system of internal membranes responsible for both respiratory and photosynthetic electron transport. They have unique food storage compounds, myxophycean starch and cyanophycin. They accomplish water-oxidizing photosynthesis by using both Photosystem I and Photosystem II (Z scheme), but under anaerobic conditions they are capable of using only Photosystem I like purple bacteria. They use same electron transport machinery for both photosynthesis and respiration. Although photoautotrophy is the major mode of nutrition in cyanobacteria,[7] some species exhibit a photoheterotrophic mode[8] by utilizing glucose as a carbon and energy source in the dark. The morphology of cyanobacteria varies from unicellular to filamentous or colonial forms. The colonies are often surrounded by a mucilaginous or gelatinous sheath, depending on environmental conditions. *Nostoc*, a filamentous cyanobacterium may produce spherical colonies as much as 3 or 4 cm in diameter. Some of the filamentous cyanobacteria have three types of cells namely: vegetative cells, climate-resistant akinetes and thick-walled heterocysts. Cyanobacteria inhabit almost all the habitats on earth; from bare rock to soil and from water to air. Although they are

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in the main free living, symbioses of some species with plant and animal species are not uncommon.

Cyanobacteria have long been known for their ecological and agricultural impact; that is, as the primary colonizers of an ecosystem, their ability to fix atmospheric nitrogen and solubilize phosphates. In recent years, significant emphasis has been given to cyanobacterial biotechnology.[9] Worldwide attention is drawn toward cyanobacteria for their possible role to help and harm humankind. Nowadays, these organisms are under consideration as an alternative source of energy because of their capabilities for generating hydrogen and ethanol. Products from some species (*Aphanizomenon flos-aquae* and *Arthospira platensis*) are available as supplements to provide a protein-rich diet. During recent decades, cyanobacteriologists have started to pay attention to the bioactivity of cyanobacteria.[10,11] A literature study of Journal of Natural Products (January 2007–October 2008), revealed the discovery of 38 new compounds from cyanobacteria among which 8 are antiprotozoal, 7 are antibacterial, 2 are antiviral, 6 are cytotoxic, 7 are protease inhibitors, 1 is a $Ca^{2+}$ channel inhibitor and the remainder (7) show no bioactivity (Figure 1). The high degree of diversity in the bioactivities of cyanobacteria is due to the broad spectrum of its secondary metabolites. As cyanobacteria do not produce a particular class of chemicals, its secondary metabolite spectrum includes 40.2% lipopeptides, 5.6% amino acids, 4.2% fatty acids, 4.2% macrolides, 9.4% amides and others.[12] The wide spectrum of cyanobacterial secondary metabolites can be explained on the basis of their ubiquitous occurrence and long evolutionary history. These metabolites exhibit a wide range of bioactivities that include some that may be relevant to the natural environment, such as antibacterial, antifungal, antiviral, cytotoxic, and others whose natural function may not be clear but may be important medicinally as anticancer agents, immunomodulators or protease inhibitors. Thus, these bioactive molecules simultaneously indicate the pharmaceutical potential of cyanobacteria.[13,14] Genome sequencing studies of cyanobacteria show significant diversity and novelty of genes responsible for bioactive proteins, ribosomal and non-ribosomal peptides, and peptide–polyketide hybrid molecules.[15]

The presence of non-ribosomal peptide synthetase and polyketide synthetase genes reveal the potential for finding novel natural product drug leads from these organisms.[16] It is well known that the non-ribosomal peptide synthetase–polyketide synthetase system produces a diverse family of compounds having biological and pharmacological properties; for example, the antibiotic—vancomycin, immunosuppressive agent—cyclosporine and anticancer agent—bleomycin.[17] In this review, an attempt has been made to focus on the natural compounds from cyanobacteria, which have shown potent activity *in vivo* or *in vitro,* and have promise to be developed as therapeutic agents.

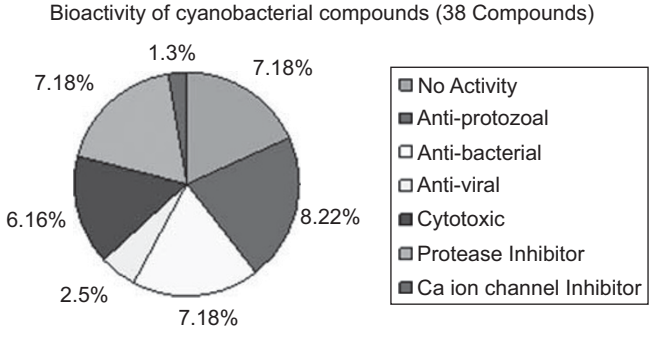



**Figure 1** Bioactivity of cyanobacterial compounds. A full color version of this figure is available at the *Journal of Antibiotics* online

## ANTICANCER ACTIVITY

There is an urgent need of new anticancer drugs because tumor cells are developing resistance against currently available drugs, like vinca alkaloids and taxanes, which is thought to be a major cause of failure in the chemotherapeutic treatment of cancers. In addition, the incidence of new types of cancer such as gliobastoma is increasing rapidly. As a result, cancer is still among the leading cause of mortality worldwide. According to an estimate from the American Cancer Society,[18] 7.6 million people died from cancer in the world during 2007. The screening of cyanobacterial extracts for new anticancer compounds was initiated in the laboratory of Moore (Oregon State University) and Gerwick (University of Hawaii) in the 1990s. The following are few examples of promising anticancer cyanobacterial metabolites with established mechanisms of action (Table 1).

Cryptophycins are potent anticancer agents produced by the cyanobacteria. Cryptophycin 1 was isolated from *Nostoc* sp. *GSV224* in Moore's lab[19] as anticancer agent. It has an $IC_{50}$ of 5 pg $ml^{-1}$ against KB human nasopharyngeal cancer cells, and 3 pg $ml^{-1}$ against LoVo human colorectal cancer cells, and thus it was found to be 100–1000 times more potent than currently available anticancer drugs; for example, taxol or vinblastine. It also exhibits anticancer activity against adriamycin-resistant M 17 breast cancer and DMS 273 lung cancer cell lines. It is a highly potent suppressor of microtubule dynamics and blocks the cells in G2/M phase. There are several analogs of cryptophycin either naturally isolated or chemically synthesized. Cryptophycin-52 (LY 355073), a chemical analogue of cryptophycin 1, entered a clinical trial but produced only marginal activity.[20] Currently two other analogues, cryptophicins 249 and 309, with improved stability and water solubility are being considered as second-generation clinical candidates.[21] The enhanced efficacy of these compounds compared with that of Cr-52 (LY 355073) has empowered new efforts to move these compounds into clinical trials.[22]

Curacin A, first isolated from *Lyngbya majuscula* by Gerwick *et al.*,[23] appeared to be a potent tubulin interactive compound but was so insoluble that its bioactivity could not be demonstrated in *in vivo* animal models. To improve on the solubility of natural curacin A, a number of soluble semi-synthetic derivatives have been generated using combinatorial chemical techniques. Currently, these compounds are undergoing preclinical evaluation as potential future anticancer drugs.

Dolastatin 10, which was originally isolated in very low quantity from the sea hare *Dolabella auricularia*, is actually a cyanobacterial metabolite, as confirmed by its direct isolation from a *Symploca* sp.[24] It is a pentapeptide containing four unique amino acids, dolavaline, dolaisoleucine, dolaproline and dolaphenine. It is a potent antiproliferative agent with an $ED_{50}$ of $4.6\times10^{-5}$ μg $ml^{-1}$. It binds to tubulin on the rhizoxin-binding site and affects microtubule assembly arresting the cell into $G_2$/M phase. Unfortunately, in clinical tests, its phase II trial as a single agent was discontinued because of the development of peripheral neuropathy in 40% of patients and the lack of significant activity in patients with hormone refractory metastatic adenocarcinoma[25] and recurrent platinum sensitive adenocarcinoma.[26] An analogue of dolastatin 10, TZT-1027 (auristatin PE or soblidotin), which differs only in the absence of the thiazoline ring from the dolaphenine residue, was found to be effective in two human xenograft models, MX-1 breast carcinoma and LX-1 lung carcinoma in mice.[27] It showed equivalent efficacy against both p53 normal and mutant cell lines.[28] Bhaskar *et al.*[29] demonstrated that a conjugate of auristatin with a monoclonal antibody directed to the adhesion molecule E-selectin inhibited the growth of prostate cancer cells by up to 85% in a mouse model.

**Table 1 Some anticancer compounds from cyanobacteria**

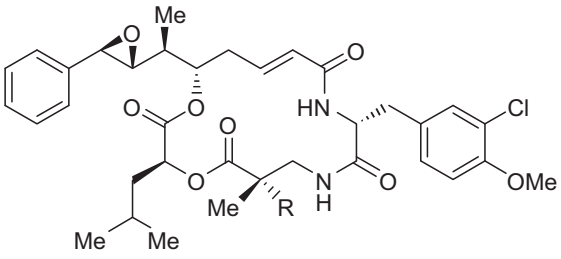
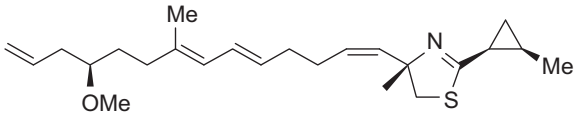
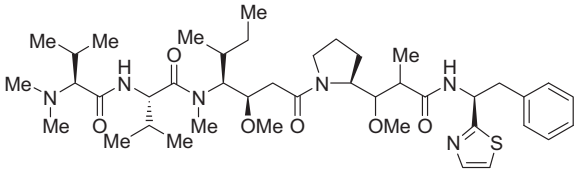
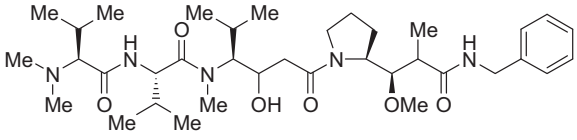
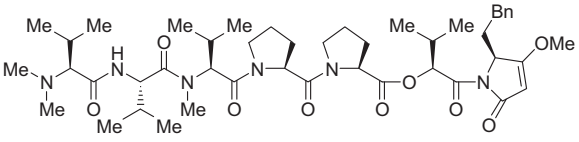
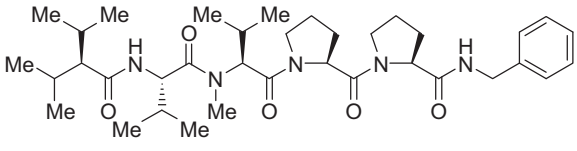
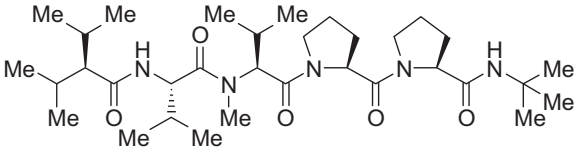
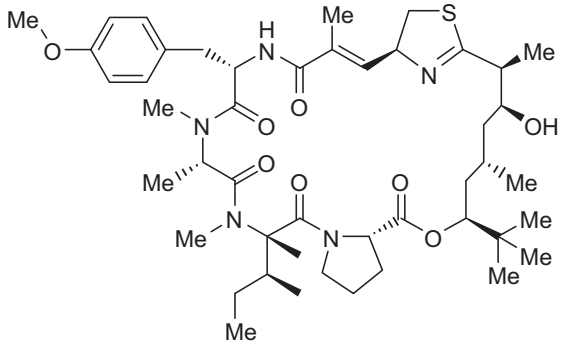
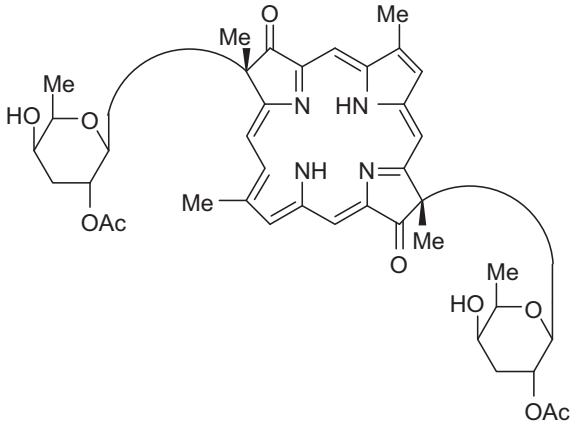
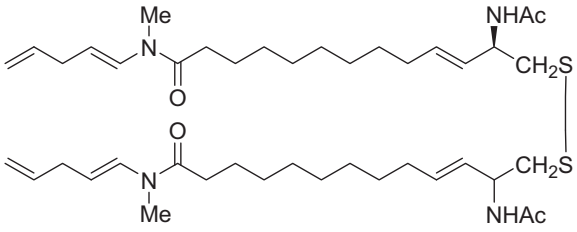
| *Compounds* | *Source* | *Active against* | *Chemical structure* |
|---|---|---|---|
| Cryptophycin-1 | *Nostoc* sp. GSV 224 | Adriamycin-resistant M 17 breast cancer and DMS 273 lung cancer cell lines |  Crytophycin 1: R=H Crytophycin 52: R=$CH_3$ |
| Cryptophycin-52 (LY 355073) | Analogue of *Cryptophycin 52* | | |
| Curacin A | *Lyngbya majuscula* | Inhibits microtubule assembly |  |
| Dolastatin 10 | *Symploca* sp. | Binds to tubulin on rhizoxin-binding site and affects microtubule assembly |  |
| TZT-1027 | Analogue of Dolastatin-10 | Effective against MX-1 breast carcinoma and LX-1 lung carcinoma in both p53 normal and mutant cells |  |
| Dolastatin 15 | *Lyngbya* sp. | Breast cancers (binds directly to vinca alkaloid site of tubulin) |  |
| Cematodin (LU-103793) | Analogue of Dolastatin-15 | |  |
| ILX-651 (Synthadotin) | Analogue of Dolastatin-15 | |  |

**Table 1 Continued**

| *Compounds* | *Source* | *Active against* | *Chemical structure* |
|---|---|---|---|
| Apratoxin A | *Lyngbya* sp. | Early stage adenocarcinoma (It induces G-1 phase cell cycle arrest) |  |
| Tolyporphin | *Tolypohrix nodosa* Bharadwaja (UH strain HT-58-2) | EMT-6 tumor cells in mouse (acts as photosensitizers) |  |
| Somocystinamide A | *Lyngbya majuscula*/ *Schizothrix* sp. Assemblage | Cyto toxic against mouse neuro-2a neuroblastoma cells |  |

Another member of the dolastatin family, dolastatin 15, is a linear peptide having an $ED_{50}$ of $2.4\times10^{-3}$ μg ml$^{-1}$ against various cancer cell lines. It binds directly to the vinca alkaloid site on tubulin and blocks the transition into M phase. No clinical trials have been undertaken with this compound because of its structural complexity, low synthetic yield and poor water solubility. Cematodin (LU-103793), a water-soluble analogue of dolastatin 15, which has a terminal benzylamine moiety in place of dolapyrolidone, retains high cytotoxicity *in vitro*. It was found to be effective in a phase I trial for treatment of breast and other cancers by BASF Pharma (Varanasi, India), but a phase II trial was discontinued following unexpected results. Currently, ILX-651 (Synthadotin), a third-generation analogue with a terminal tert-butyl moiety in place of dolapyrolidone, has successfully completed a phase I clinical trial and a phase II trial has been recommended.[30]

Apratoxin A is a cyclodepsipeptide isolated from a *Lyngbya* sp. collected from Guam. This compound is cytotoxic to human tumor cell lines with $IC_{50}$ values ranging from 0.36 to 0.52 nM *in vitro*,[31] but showed only marginal activity against early stage adenocarcinoma *in vivo*. It induces G-1 phase cell cycle arrest and apoptosis.[32]

The photosynthetic pigment C-PC is reported to have various pharmacological characteristics including anti-inflammatory and anticancer activities because of its β-subunit. The recombinant β-subunit of C-PC has been demonstrated to have anticancer properties. Recombinant C-PC/β, tested on four different cell lines exhibited a high rate of proliferation inhibition and apoptotic induction. It has been shown that the recombinant protein interacts with membrane-associated β-tubulin and glyceraldehyde-3-phosphate dehydrogenase. In addition, the nuclear level of glyceraldehyde-3-phosphate dehydrogenase decreased significantly.[33] These properties suggest that C-PC/β may have promise as a cancer prevention or therapy agent.

Tolyporphin from *Tolypothrix nodosa* exhibits a potent photosensitizing activity against tumor cells and is 5000 times more effective than

**Table 2 Antiviral compounds isolated from cyanobacteria**

| *Compound* | *Source* | *Biological activity against* | *Chemical structure* |
|---|---|---|---|
| Spirulan | *Spirulina* sp. | HIV-1 and HIV-2 (inhibit reverse transcriptase) HSV, influenza | *Sulphated polysaccharide composed of O*-rhamnosyl-acofriose and *O*-hexuronosylrhamnose |
| Nostoflan | *Nostoc flagilliforme* | HSV-1 (HF), HSV-2 (UW-268), HCMV(Towne) Influenza (NWS), Adeno (type 2), Coxsackie (Conn-5) | *→4)-D-Glcp-(1→, →6,4)-D-Glcp- (1→, → 4)-D-Galp-(1→, →4)-D-Xylp-(1→, D-GlcAp-(1→, D-Manp-(1→ with a ratio of 1:1:1:1:0.8:0.2* |
| Cyanovirin-N | *Nostoc ellipsosporum* | HIV-1 (interacts with high mannose groups of envelope glycoproteins, gp120 and blocks its interaction with target cell receptors) HIV-2 HSV-6 Mesles virus SIV FIV | *(NH2) Leu–Gly–Lys–Phe–Scr–Ghs–Thr–Cys–Tyr–Asn–Ser–Ala–Ile–Gln–Gly–Ser–Val–Len–Thr–Ser–The–Cys–Glu–Arg–Thr–Asn–Gly–Gly–Thr–Ser–The–Ser–Ser–Ilg–Asp–Leu–Asn–Ser–Val–Ile–Glu–Asn–Val–Asp–Gly–Ser–Len–Lys–Trp–Gln–Pro–Ser–Asn–Phe–Ile–Glu–Thr–Cys–Arg–Asn–Thr–Gln–Leu–Ata–Gly–Ser–Ser–Glu–Leu–Ala–Ala–Glu–Cys–Lys–Thr–Arg–Ala–Glu–Gln–Phe–Val–Ser–Thr–Lys–Ile–Asn–Leu–Asp–Asp–His–Ile–Ala–Asn–Ile–Asp–Gly–Tla–Leu–Lys–Thr–Gla (COOH)* |
| Scytovirin N | *Scytonema varium* | HIV–1 (interacts with oligosaccharides conaining α1–2, α1–2, α1–6 tetramannose units of envelope glycoproteins, gp120, gp160, gp41) | Domain-1 ($NH_2$) Ala–Ala–Ala–His–Gly–Ala–Thr–Gly–Gln–Cys–Phe–Gly–Ser–Ser–Ser–Cys–Thr–Arg–Ala–Gly–asp–Cyst–Gln–Lys–Ser–Asn–Ser–Cys–Arg–Asn–Pro–Gly–Gly–Pro–Asn–Lys–Ala–Glu–asp–Trp–Cys–Tyr–Thr–Pro–Gly–Lys–Pro–<br>Domain-2 *Gly–Pro–Asp–Pro–Lys–Arg–Ser–Thr–Gly–Gln–Cys–Phe–Gly–Ser–Ser–Ser–Cys–Thr–Arg–Ala– Gly–asp–Cys–Gln–Lys–Asn–Asn–Ser–Cys–Arg–Asn–Pro–Gly–Gly–Pro–Asn–Asn–Ala–Glu–Asn–Trp–Cys–Tyr–Thr–Pro–Gly–Ser–Gly (COO*H) |
| Sulfoglycolipid | *Scytonema* sp. | HIV-1 (inhibit reverse transcriptase and DNA polymerases) | Sulfoquinovosyldiacylglyerols |

Abbreviation: HIV, human immunodeficiency virus.

the photodynamic treatment (photofrin II).[34] Somocystinamide A (ScA) was isolated from the marine cyanobacterium *Lyngbya majuscula*. It is a pluripotent inhibitor of angiogenesis and tumor cell proliferation. It induces apoptosis in tumor and angiogenic endothelial cells. *In vitro*, picomolar concentrations of somocystinamide A are sufficient to disrupt proliferation and tubule formation in endothelial cells.[35] In addition, there are numerous other anticancer cyanobacterial metabolites that have not been covered here as their mechanisms of action have yet to be determined.

## ANTIVIRAL ACTIVITY

The global spread of deadly viral diseases like HIV–acquired immune deficiency syndrome and dengue may have dramatic consequences. New potent and safe antiviral agents are urgently needed in this situation. Presently, the only approved anti-HIV treatment (HAART (highly active antiretroviral therapy) tri therapy), which is effective in controlling the progression of HIV infections has proved to be toxic, to induce strong viral resistance, and is unable to eradicate the causative viral agent.[36] The following are three classes of cyanobacterial compounds with potent *in vitro* antiviral activity. Their structures are depicted in Table 2.

### Polysaccharides

The most significant antiviral cancer polysaccharides are spirulan and Ca-spirulan from *Spirulina* sp. These compounds from the extracts of cyanobacteria showed potent and broad-spectrum activity against HIV-1, HIV-2, H, influenza and a series of other enveloped viruses. They inhibit the reverse transcriptase activity of HIV-1 (like azidothymidine). These sulfated polysaccharides prevent virus-cell attachment and fusion with host cells. They also inhibit the fusion between HIV-infected and uninfected CD4+ lymphocytes, a mechanism that greatly enhances viral infectivity. These compounds have advantages as antiviral agents over other sulfated polysaccharides because of reduced anticoagulant properties.[37] Also noteworthy is nostoflan, an acidic polysaccharide from *Nostoc flagelliforme* that exhibits potent virucidal activity against herpes simplex virus-1 (ref. 38).

### Carbohydrate-binding proteins

Currently, two carbohydrate-binding proteins namely cyanovirin-N and scytovirin are being developed as potent virucidal drugs. These carbohydrate-binding proteins show antiviral activity by interfering with multiple steps in the viral fusion process.

Cyanovirin-N is an 101 amino acid long, 11 kDa polypeptide isolated from *Nostoc ellipsosporum* showing potent *in vitro* and *in vivo* activity against HIV and other lentiviruses in nanomolar concentrations.[39] It interferes with the binding of HIV gp120 proteins with CD4+ receptors and the chemokine CCR5 or CXCR4 co-receptors of target cells, and thus, inhibits fusion of HIV virus with CD4 cell membrane. It is being developed as a vaginal gel for inhibiting the sexual transmission of HIV by Cellegy Pharmaceuticals, San Francisco, CA, USA. The development of a vaginal microbicide would be of major benefit for reducing the global spread of HIV-1 (ref. 40). It also inhibits herpes simplex virus-6 and measles virus *in vitro*.[41]

Scytovirin is a 95 amino acid long, 9.7 kDa polypeptide containing five intra chain disulphide bonds. It was first isolated from the aqueous extract of *Scytonema varium*.[42] It binds to the envelope glycoprotein of HIV (gp120, gp160 and gp41) and inactivates the virus in low nanomolar concentrations. NMR analysis revealed that scytovirin has two domains and the first domain (a.a. 1–48) has similar anti-HIV activity to the full-length scytovirin.[43]

In addition, two cyclic depsipeptides, ichthyopeptins A and B, were also isolated from *Microcystis ichthyoblabe*. They show antiviral activity against influenza A virus with an $IC_{50}$ value of 12.5 μg $ml^{-1}$ (ref. 44).

**Table 3 Some antibacterial compounds recently isolated from cyanobacteria**

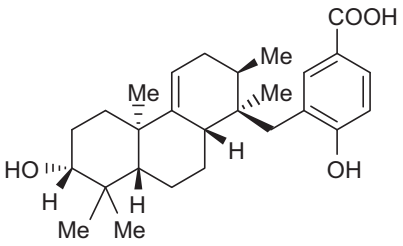
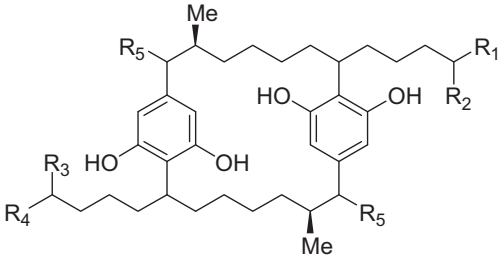
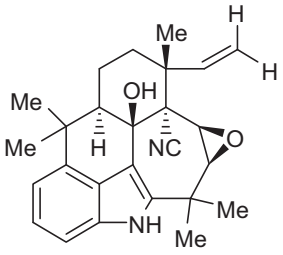
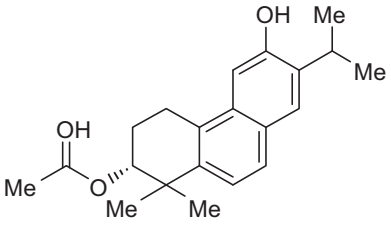
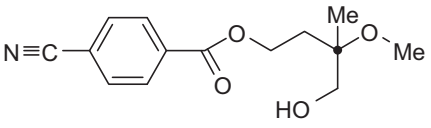
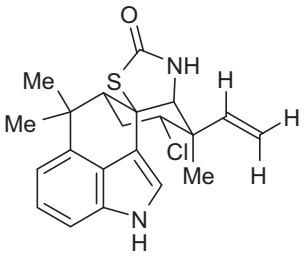
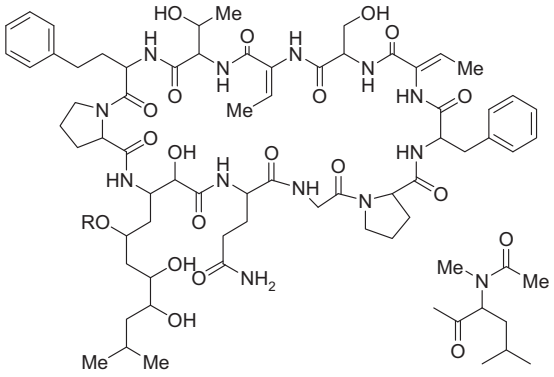
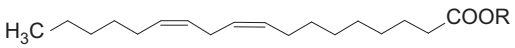
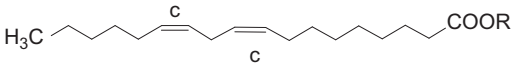
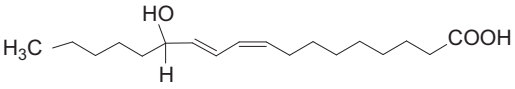
| *Compound* | *Source* | *Biological activity against* | *Chemical structure* | *Ref.* |
|---|---|---|---|---|
| Noscomin | *Nostoc commune* | *Bacillus cereus, Staphylococcus epidermidis, Escherichia coli* |  | Jaki *et al*.[50] |
| Carbamido-cyclophanes[a] | *Nostoc* sp. CAVN 10 | *Staphylococcus aureus* |  | Bui *et al*.[51] |
| Ambiguine I isonitrile | *Fischrella* sp. | *E. coli* ESS K-12, *Staphyloccocus albus*, *Bacillus subtilis* |  | Raveh and Carmeli[52] |
| Norbietane diterpenoid (20-nor-3α-acetoxyabieta-5,7,9,11,13-pentaene) | *Microcoleus lacustris* | *S. aureus* |  | Gutierrez *et al*.[53] |
| Phenolic compound | *Nostoc muscorum* | *B. subtilis*, *B. cereus*, *E. coli*, *Salmonella* Typhi, *S. aureus* |  | El-Sheekh *et al*.[54] |
| Hapalindole T | *Fischerella* sp. | *S. aureus*, *Pseudomonas aeruginosa*, *Salmonella* Typhi, *E. coli* |  | Asthana *et al*.[55] |

**Table 3 Continued**

| *Compound* | *Source* | *Biological activity against* | *Chemical structure* | *Ref.* |
|---|---|---|---|---|
| Pahayokolide A | *Lyngbya* sp. | *Bacillus* sp. |  | Berry *et al.*[56] |
| Fatty acids | *Oscillatoria redeki HUB051* | *B. subtilis* SBUG 14, *Micrococcus flavus* SBUG 16, *S. aureus* SBUG 11, *S. aureus* ATCC 25923 |  α-dimorphecolic acid<br>  coriolic acid<br>  13-hydroxy-9Z, 11E-octadeca di enoic acid (13-HODE) | Mundt *et al.*[57] |

[a]Carbamidocyclophanes: Carbamidocyclophane A: **R1**, Cl; **R2**, Cl; **R3**, Cl; **R4**, Cl; **R5**, $OCONH_2$; Carbamidocyclophane B: **R1**, Cl; **R2**, Cl; **R3**, Cl; **R4**, H; **R5**, $OCONH_2$; Carbamidocyclophane C: **R1**, Cl; **R2**, Cl; **R3**, H; **R4**, H; **R5**, $OCONH_2$; Carbamidocyclophane D: **R1**, Cl; **R2**, HI; **R3**, H; **R4**, H; **R5**, $OCONH_2$; Carbamidocyclophane E: **R1**, H; **R2**, H; **R3**, H; **R4**, H; **R5**, $OCONH_2$.

### Sulfoglycolipids

The natural sulfoglycolipids from cyanobacteria are also reported to inhibit HIV–reverse transcriptase and DNA polymerases.[45]

## ANTIBACTERIAL ACTIVITY

If the incidence of bacterial resistance continues to rise, some diseases may become untreatable. Of late, the multi-drug-resistant bacteria causing nosocomial infections like methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci* and AmpC β-lactamase-producing Enterobacteriaceae have posed therapeutic challenges and are of great concern worldwide.[46] Therefore, searches for new antibiotics to treat bacterial infections are urgently needed. In an effort to develop new antibiotics, scientists are screening cyanobacterial extracts for their antibacterial activity[47–49] and found them potentially active against various bacteria. Unfortunately, very few antibacterial compounds from cyanobacteria have been structurally characterized to date (Table 3). Noscomin[57] from *Nostoc commune*, showed antibacterial activity against *Bacillus cereus* (MIC 32 p.p.m.), *Staphylococcus epidermidis* (MIC 8 p.p.m.), *Escherichia coli* (MIC 128 p.p.m.) comparable to that of the standard drugs. Bhateja *et al.*[57] reported the antibacterial activity of *Anabaena* extract against vancomycin-resistant *S. aureus* with an MIC of 32–64 $\mu g\,ml^{-1}$. Carbamidocyclophanes are paracyclophanes isolated from *Nostoc* sp. CAVN 10. They show moderate antibacterial activity against *Staphylococcus aureus.*[58] Raveh *et al.*[50] isolated nine ambiguines from *Fischerella* sp., which showed antimicrobial activity. Ambiguine-I isonitrile showed more potent antibacterial activity than streptomycin against *Bacillus subtilis* and *Staphylococcus albus*. Recently, two new norbietane compounds, showing antibacterial activity against *S. aureus*, *S. epidermidis*, *Salmonella* Typhi, *Vibrio cholarae*, *B. subtilis*, *B. cereus*, *E. coli* and *Klebsiela pneumoniae*, were isolated from *Micrococcus lacustris.*[51]

## ANTIPROTOZOAL ACTIVITY

According to an estimate of World Health Organization, more than one billion people throughout the world are suffering from tropical diseases caused by *Plasmodium, Trypanosoma, Leishmania, Schistosoma* and others.[59] The failures in the treatment of these diseases, especially in cases of malaria[60] and leishmaniasis,[61] are due to development of resistance by these protozoa. On the other hand, progress in the advancement of drug discovery programs against these diseases is very slow.[62] In an effort to encourage the development of effective and affordable treatment of these diseases, the Panamanian

**Table 4 Antiprotozoal compounds from cyanobacteria isolated in ICBG project**

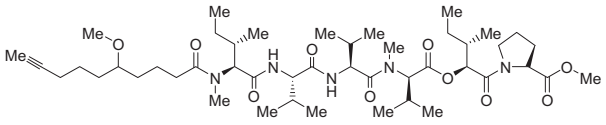
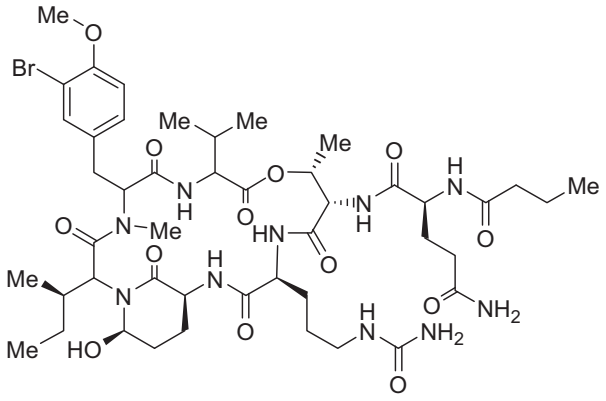
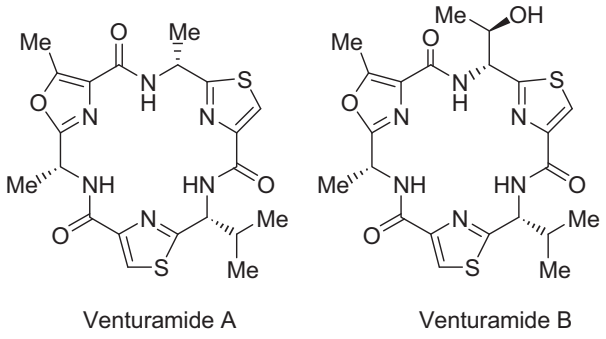
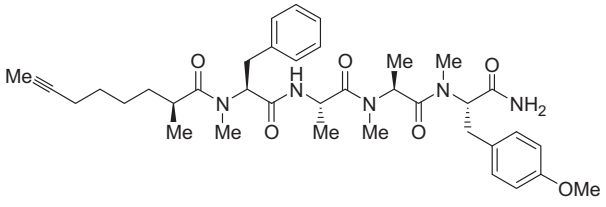
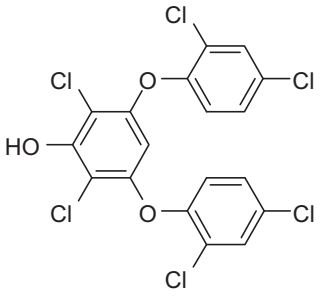
| *Compounds* | *Source* | *Active against* | *Chemical structure* | *Ref.* |
|---|---|---|---|---|
| Viridamide A | *Oscillatoria nigro virdis* | *Trypanosoma cruzi, Leishmania mexicana, Plasmodium falciparum* |  | Simmons *et al.*[59] |
| Symplocamide A | *Symploca* sp. | *T. cruzi, L. donovani, P. falciparum* |  | Linnington *et al.*[63] |
| Venturamides | *Oscillatoria* sp. | *P. falciparum* |  Venturamide A Venturamide B | Linnington *et al.*[64] |
| Dragomabin | *Lyngbya majuscula* | *P. falciparum* |  | Mcphail *et al.*[65] |
| Ambigol C | *Fischerellambigua* | *T. rhodesiense, P. falciparum* |  | Wright *et al.*[66] |

**Table 5 Some protease inhibitors isolated from cyanobacteria**

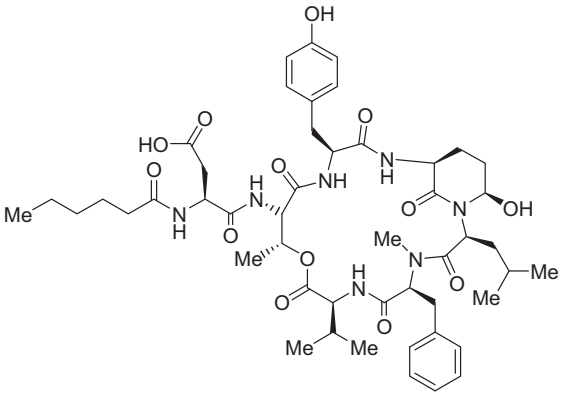
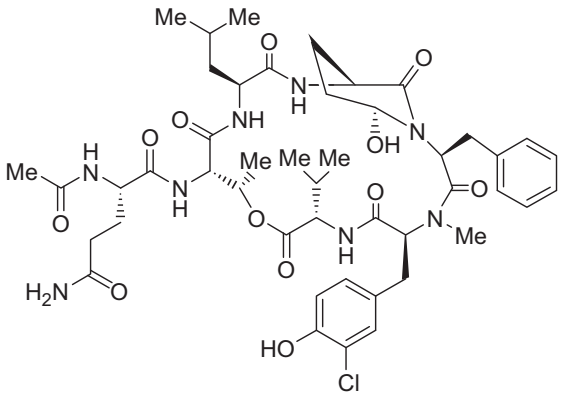
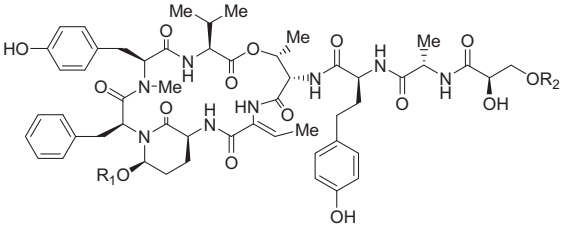
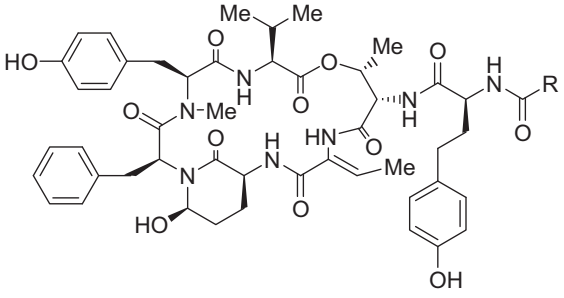
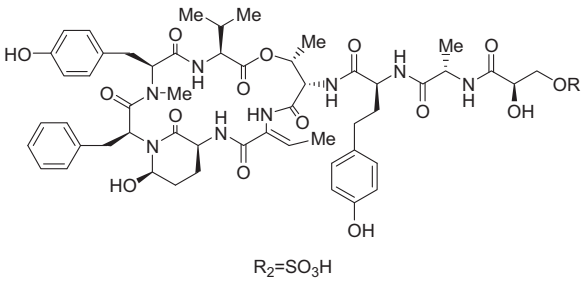
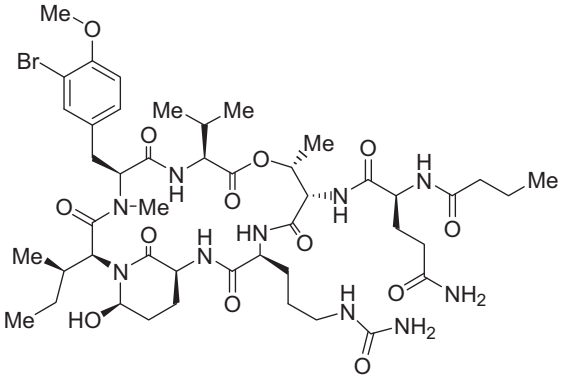
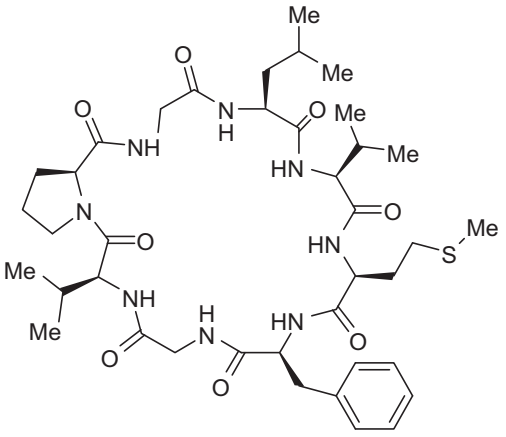
| *Compound* | *Source* | *Biological activity against* | *Chemical structure* | *Ref.* |
|---|---|---|---|---|
| Cyanopeptolin 963 A | *Microcystis PCC 7806* | Chymotrypsin |  | Bister *et al.*[70] |
| Cyanopeptolin 954 | *Microcystis aeruginosa NIVA Cya 43* | Chymotrypsin |  | Elert *et al.*[71] |
| Lyngbyastatin 5-7 | *Lyngbya* sp. | Elastase |  Lynbyastatin 5: $R_1$=H, $R_2$=H Lynbyastatin 6: $R_1$=$CH_3$, $R_2$=$SO_3Na$  Lynbyastatin 7: R=$(CH_2)_4CH_3$ | Taori *et al.*[72] |

**Table 5 Continued**

| *Compound* | *Source* | *Biological activity against* | *Chemical structure* | *Ref.* |
|---|---|---|---|---|
| Lyngbyastatin 4 | *Lyngbya confervoides* | Elastase<br>Chymotrypsin |  <br>$R_2$=$SO_3H$ | Matthew *et al.*[73] |
| Symplocamide A | *Symploca* sp. | Chymotrypsin |  | Linnington *et al.*[63] |
| Planktocyclin | Planktothrix rubescens | Trypsin, α-chymotrypsin<br>Human caspase-8 |  | Baumann *et al.*[74] |

International Co-operative Biodiversity Group is screening extracts from terrestrial and marine sources. Recently, this project has reported the isolation of five classes of antiprotozoal compounds from cyanobacteria (Table 4). In addition, the protease inhibitor, nostocarboline,[67] an alkaloid isolated from *Nostoc* sp. 78-12 A was also found to be active against *Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani* and *Plasmodium falciparum* with $IC_{50}$ values ranging from 0.5 to 0.194 μM. Aerucyclamide C[68] isolated from *Microcystis aeruginosa* PCC 7806 was also found to be active against *T. brucei*, and the already known aerucyclamide B against *P. falciparum* with submicromolar $IC_{50}$ values. Clark *et al.*[69] isolated six new acyl proline derivatives, tumonoic acids D-I, from the marine cyanobacterium *Blennothrix cantharidosmum* among which tumonoic acid I displayed moderate activity in an antimalarial assay ($IC_{50}$ 2 μM).

## PROTEASE INHIBITION ACTIVITY

The discovery of new protease inhibitors may be of great pharmaceutical value. Jaspars and Lawton[13] described some protease inhibitors of cyanobacterial origin, like microginins, aeruginosins and cyanopeptolins. Microginins are used in the treatment of high blood pressure. Serine protease inhibitors like cyanopeptolin are applied in the treatment of conditions such as asthma and viral infections. Some protease inhibitors isolated from cyanobacteria in the last 5 years are given in Table 5.

Recently, Taori *et al.*[75] reported the isolation of two kempopeptins having protease inhibitory activity. Kempopeptin A, a cyclodepsipeptide from a marine *Lyngbya* sp. exhibited an $IC_{50}$ of 0.32 μM against elastase and 2.6 μM against chymotrypsin, whereas kempopeptin B inhibited trypsin with an $IC_{50}$ of 8.4 μM.

## IMMUNOMODULATORY ACTIVITY

Hayashi *et al.*[76] investigated the effect of *Spirulina* in mice and reported increased phagocytic activity and increased antigen production in the test animals under study. Qureshi and Ali[77] reported an increased phagocytic activity, increased antigen production and increased natural killer cell-mediated antitumor activity in chicken for the same cyanobacterium (*Spirulina*). In a preliminary small clinical study, an increase in 13.6-fold in interferon and 3-fold in interleukin (IL)-1β and -4 was observed in human blood cells incubated with *Spirulina* extracts.[78] Khan *et al.*[79] reported that different products prepared from *Spirulina* influence immune systems in various ways such as increasing the phagocytic activity of macrophages, stimulating antibody and cytokine production, increasing accumulation of natural killer cells into tissues and activating T and B cells. In a clinical study in Korea,[80] a significant rise in plasma IL-2 concentration, and a significant reduction in IL-6 concentration in humans was observed after the consumption of *Spirulina* at home, 8 g $day^{-1}$, for 16 consecutive weeks. Although *Spirulina* was found to be safe, other species of cyanobacteria contain metabolites, such as microcystin, that are cytotoxic to lymphocytes and inhibit membrane-bound leucine aminopeptidase.[81] The latter appears to be linked to antigen-processing and antigen presentation response.[82]

The immunotoxicity of a cyanobacterial bloom extract containing microcystin was reported for the first time by Shen *et al.*[83] Treatment with cyanobacterial bloom extract resulted in the inhibition of lipopolysaccharide-induced lymphoproliferation and the dose-dependent decrease in the numbers of antibody-forming cells in mice that were immunized by using T-dependent antigen sheep red blood cells. Thus, exposure to cyanobacterial bloom extract resulted in immunosuppression in mice. Shi *et al.*[84] evaluated the effect of cyanobacterial bloom extract containing microcystins on the expression of multiple cytokines, including proinflammatory (IL-1β, tumor necrosis factor-α and IL-6) and Th1/Th2-related cytokines (IL-2, IL-4 and IL-10) *in vivo*. They observed the distinct patterns of expression of these cytokines suggesting a modulation of cytokine networks.

## CONCLUSIONS

Cyanobacteria constitute a unique group of oxygenic photosynthetic bacteria and populate diverse habitats throughout the world. Their potential as a good source of new therapeutic lead compounds has been realized during the last two decades. We have discussed several bioactive molecules obtained from cyanobacteria showing a broad spectrum of activities, such as antitumor, antibacterial and antiviral effects and protease inhibition. Another advantage of cyanobacteria as a microbial source for drug discovery lies in the economy of their cultivation compared with other microorganisms, as they require only simple inorganic nutrients for growth. Thus, it seems that the cyanobacteria have the potential for expanded utilization in drug discovery.

Despite their potent biological activities, very few cyanobacterial compounds have entered clinical trials and no cyanobacterially derived compound has been approved by the Food and Drug Administration. In our opinion, the pharmaceutical potential of cyanobacteria deserves more scientific attention and interdisciplinary research. Further, to find novel compounds, cyanobacterial strains from still unexplored and extreme habitats should also be studied.

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1 Fleming, A. On the antibacterial action of cultures of a penicillium, with special reference to their use in the isolation of *B. influenzæ*. *Br. J. Exp. Pathol.* **10,** 226–236 (1929).
2 Lam, K. S. New aspects of natural products in drug discovery. *Trends Microbiol.* **15,** 279–289 (2007).
3 Newman, D. J. *et al.* Natural products as sources of new drugs over the period 1981–2002. *J. Nat. Prod.* **66,** 1022–1037 (2003).
4 Adams, C. & Brantner, V. Estimating the cost of new drug development: is it really 802 million dollars? *Health Aff. (Millwood)* **25,** 420–428 (2006).
5 Liu, X. J. & Chen, F. Cell differentiation and colony alteration of *Nostoc flagelliforme*, an edible terrestrial cyanobacterium in different liquid suspension culture. *Folia Microbiol.* **48,** 619–625 (2003).
6 Schopf, W. & Packer, B. Early archaen microfossils from warrawoona group, Australia. *Science* **237,** 70–73 (1987).
7 Stal, L. J. & Moezelaar, R. Fermentation in cyanobacteria. *FEMS Microbiol. Rev.* **21,** 179–211 (1997).
8 Rippka, R. Photoheterotrophy and chemoheterotrophy among unicellular blue- green algae. *Arch. Microbiol.* **87,** 93–98 (1972).
9 Thajuddin, N. & Subramanian, G. Cyanobacterial biodiversity and potential application in biotechnology. *Curr. Sci.* **89,** 47–57 (2005).
10 Singh, S., Kate, B. N. & Banerjee, U. C. Bioactive critical compounds from cyanobacteria and microalgae: an overview. *Crit. Rev. Biotechnol.* **25,** 73–95 (2005).
11 Gademann, K. & Portmann, C. Secondary metabolites from cyanobacteria: complex structures and powerful bioactivities. *Curr. Org. Chem.* **12,** 326–341 (2008).
12 Burja, A. M., Banaigs, B., Abou-Mansour, E., Burgess, J. G. & Wright, P. C. Marine cyanobacteria- a prolific source of natural products. *Tetrahedron* **57,** 9347–9377 (2001).
13 Jaspars, M. & Lawton, L. A. Cyanobacteria- a novel source for pharmaceuticals. *Curr. Opin. Drug Dis. Dev.,* **1,** 77–84 (1998).
14 Devillers, J. *et al.* Prediction of biological activity profiles of cyanobacterial secondary metabolites. *SAR QSAR. Environ. Res.* **18,** 629–643 (2007).
15 Sivonen, K. & Börner, T. Bioactive compounds produced by Cyanobacteria. In *The Cyanobacteria: Molecular Biology, Genomics and Evolution*: (eds Herrero A, Flores E). (Caister Academic Press, Hethersett, UK, 2008).
16 Ehernich, I. M., Waterbury, J. B. & Webb, E. A. Distribution and diversity of natural product genes in marine and fresh water cyanobacterial cultures and genomes. *App. Env. Microbiol.* **71,** 7401–7403 (2005).
17 Schwarjer, D., Finking, R. & Marachiel, M. A. Nonribosomal peptides: from genes to products. *J. Nat. Prod. Rep.* **20,** 275–287 (2003).
18 American Cancer Society (December 2007). Report sees 7.6 million global 2007 cancer deaths. Reuters, http://uk.reuters.com/article/2007/12/17/health-cancer-world-dc-idUKN1633064920071218.
19 Patterson, G. M. L. *et al.* Antineoplastic activity of cultured blue-green algae (Cyanophyta). *J. Phycol.* **27,** 530–536 (1991).
20 Shin, C. & Teicher, B. A. Cryptophycins: a novel class of potent antimititotic antitumor depsipeptide. *Curr. Pharmaceutical Design* **13,** 1259–1276 (2001).
21 Liang, J. *et al.* Cryptophycin-309 249 and other cryptophycins analogs: preclinical efficacy studies with mouse and human tumors. *InVest. New Drugs* **23,** 213–224 (2005).
22 Yousong, D., Seufert, H., Zachary, Q. B. & David, H. S. Analysis of the cryptophycin P450 epoxidase reveals substrate tolerance and cooperativity. *J. Am. Chem. Soc.* **130,** 5492–5498 (2008).
23 Gerwick, W. H. *et al.* Structure of curacin a, a Novel antimitotic, antiproliferative and brine shrimp toxic natural product from the marine cyanobacterium. *Lyngbya Majuscula*. *J. Org. Chem.* **59,** 1243–1245 (1994).
24 Leusch, H., Moore, R. E., Paul, V. J., Mooberry, S. L. & Corbett, T. H. Isolation of dolastatin 10 from the marine cyanobacterium Symploca sp. VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. *J. Nat. Prod.* **64,** 907–910 (2001).
25 Vaishampayan, U. *et al.* Phase II Study of dolastatin 10 in patients with hormone-refractory metastatic prostate adenocarcinoma. *Clin. Cancer Res.* **6,** 4205–4208 (2000).
26 Hoffman, M., Blessing, J. & Lentz, S. A phase II trial of dolastatin-10 in recurrent platinum-sensitive ovarian carcinoma: a Gynecologic Oncology Group study. *Gynecol. Oncol.* **89,** 95–98 (2003).
27 Kobayashi, M. *et al.* Antitumor activity of TZT-1027, a novel dolastatin 10 derivative. *Jpn. J. Cancer Res.* **88,** 316–327 (1997).
28 Natsume, T. *et al.* Antitumor activity of TZT-1027 (Soblidotin) against vascular endothelial growth factor secreting human lung cancer *in vivo*. *Cancer Sci*. **94,** 826–833 (2003).

29 Bhaskar, V. *et al.* E-selectin up-regulation allows for targeted drug delivery in prostate cancer. *Cancer Res.* **63,** 6387–6394 (2003).
30 Cunningham, C. *et al.* Phase I and pharmacokinetic study of the dolastatin-15 analogue tasidotin (ILX 651) administerd intravenously on days 1, 3 and 5 every 3 weeks in patients with advanced solid tumors. *Clin. Cancer Res.* **11,** 7825–7833 (2005).
31 Leusch, H., Yoshida, W. Y., Moore, R. E., Paul, V. J. & Corbett, T. H. Total structure determination of apratoxin a, a potent novel cytotoxin from the marine cyanobacterium *Lyngbya majuscula*. *J. Am. Chem. Soc.* **123,** 5418–5423 (2001).
32 Leusch, H. *et al.* A functional genomics approach to the mode of action of Apratoxin A. *Nat. Chem. Biol.* **2,** 158–166 (2006).
33 Wang, H., Liu, Y., Gao, X., Carter, C. L. & Liu, Z. R. The recombinant β subunit of c-phycocyanin inhibits cell proliferation and induces apoptosis. *Cancer Lett.* **247,** 150–158 (2007).
34 Morliere, P. *et al.* Tolyporphin; a natural product from cyanobacteria with potent photosensitizing activity against tumor cells *in vitro* and *in vivo*. *Cancer Res.* **58,** 3571–3578 (1998).
35 Wrasidlo, W. *et al.* The marine lipopeptide Somocystinamide A triggers apoptosis via caspase 8. *Appl. Biol. Sci.* **10,** 2313–2318 (2008).
36 Luescher-Mattli, M. Algae as a possible source of new antiviral agents. *Curr. Med. Chem. Anti-infect. Agents* **2,** 219–225 (2003).
37 Feldmann, S. C., Reynaldi, S., Stortz, C. A., Cerezo, A. S. & Damont, E. B. Antiviral properties of fucoidan fractions from Leathesia difformis. *Phytomedicine* **6,** 335–340 (1999).
38 Kenji, L. K. *et al.* Isolation of an antiviral polysacharide, nostoflan, from a terrestrial cyanobacteium, *Nostoc flagilliforme*. *J. Nat. Prod.* **68,** 1037–1041 (2005).
39 Boyd, M. R. *et al.* Discovery of cyanovirin-N, a novel human immunne deficiency virus inactivating protein that binds viral surface envelope glycorotein gp120: potential application to microbicide development. *Antimicrob. Agents Chemother.* **41,** 1521–1530 (1997).
40 Klasse, P. J., Shattock, R. & Moore, J. P. Antiretroviral drug—based microbicides to prevent HIV-1 sexual transmission. *Ann. Rev. Med.* **59,** 455–471 (2008).
41 Dey, B. *et al.* Multiple antiviral activities of cyanovirin-N: blocking HIV type1 gp120 interaction with CD4 co receptor and inhibition of diverse enveloped viruses. *J. Virol.* **74,** 4562–4569 (2000).
42 Bokesch, H. R. *et al.* A potent novel anti-HIV protein from the cultured cyanobacterium. *Scytonema Varium. Biochem.* **42,** 2578–2584 (2003).
43 Xiong, C., O'Keefe, B. R., Byrd, R. A. & McMohan, J. B. Potent anti-HIV activity of scytovirin domain 1 peptide. *Peptides* **27,** 1668–1675 (2006).
44 Zainuddin, E. N. *et al.* Cyclic depsipeptides, ichthyopeptins A and B, from Microcystis ichthyoblabe. *J. Nat. Prod.* **70,** 1084–1088 (2007).
45 Loya, S. *et al.* The inhibition of the reverse transcriptase of HIV-1 by the natural sulfoglycolipids from cyanobacteria: contribution of different moieties to their high potency. *J. Nat. Prod.* **61,** 891–895 (1998).
46 Reinert, R., Donald, E. L., Rosi, F. X., Watal, C. & Dowzicky, M. Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the *in vitro* activity of tigecycline. *J. Antimicrob. Chemother.* **60,** 1018–1029 (2007).
47 Kreitlow, S., Mundt, S. & Lindequist, U. Cyanobacteria- a potential source of new biologically active substance. *J. Biotechnol.* **70,** 61–73 (1999).
48 Skulberg, O. M. Microalgae as a source of bioactive molecules; experience from cyanophyte research. *J. App. Phycol.* **12,** 341–348 (2000).
49 Biondi, N. *et al.* Cyanobacteria from benthic mats of Antarctic lakes as a new source of bioactivities. *J. Appl. Microbiol.* **105,** 105–115 (2008).
50 Jaki, B., Orjala, J. & Sticher, O. A novel extracellular diterpenoid with antibacterial activity from the cyanobacterium. *Nostoc Comm. J. Nat. Prod.* **62,** 502–503 (1999).
51 Bui, T. N., Jansen, R., Pham, T. L. & Mundt, S. Carbamidocyclophanes A-E, chlorinated paracyclophanes with cytotoxic and antibiotic activity from the vietnamese cyanobacterium. *Nostoc sp. J. Nat. Prod.* **70,** 499–503 (2007).
52 Raveh, A. & Carmeli, S. Antimicrobial ambiguines from the cyanobacterium Fischerella sp. collected in Israel. *J. Nat. Prod.* **70,** 196–201 (2007).
53 Gutierrez, R. M. P., Flores, A. M., Solis, R. V. & Jimenez, J. C. Two new antibacterial norbietane diterpenoids from cyanobacterium. *Micrococcus lacustris. J. Nat. Med.* **62,** 328–331 (2008).
54 El-Sheekh, M. M., Osman, M. E. H., Dyab, M. A. & Amer, M. S. Production and characterization of antimicrobial active substance from cyanobacterium. *Nostoc muscorum. Env. Toxicol.* **21,** 42–50 (2006).
55 Asthana, R. K. *et al.* Identification of an antimicrobial entity from Fischerella sp. colonizing neem tree bark. *J. App. Phycol.* **18,** 33–39 (2006).
56 Berry, J., Gantar, M., Gawley, R. E., Wang, M. & Rein, K. S. Pharmacology and toxicology of phayokolide A, a bioactive metabolite from a fresh water species of Lyngbya isolated from the Florida everglades. *Comp. Biochem. Physiol, C,* **139,** 231–238 (2004).
57 Mundt, S., Kreitlow, S. & Jansen, R. Fatty acids with antibacterial activity from the cyanobacterium *Oscillatoria redekei HUB051*. *J. Appl. Phycol.* **15,** 263–267 (2003).
58 Bhateja, P., Mathur, T., Pandya, M., Fatma, T. & Rattan, A. Activity of blue- green microalgae extracts against *in vitro* generated *Staphylococcus aureus* with reduced susceptibility to vancomycin. *Fitoterpia* **77,** 233–235 (2006).
59 Simmons, L. T. *et al.* Viridamides A and B, lipodepsipeptides with antiprotozoal activity from marine cyanobacterium. *Oscillatoria Nigro Viridis. J. Nat. Prod.* **71,** 1544–1550 (2008).
60 Lanzer, M. R. & Rohrbach, P. Subcellular pH and Ca2+ in Plasmodium falciparum: implications for understanding drug resistance mechanisms. *Curr. Sci.* **92,** 1561–1570 (2007).
61 Prioto, G. *et al.* Nifurtimox- eflornithine combination therapy for second-stage *Trypanosoma brucei* gambiense sleeping sickness: a randomized clinical trial in Congo. *Clin. Infec. Dis.* **45,** 435–1444 (2007).
62 Sheifert, K., Lunke, A., Croft, S. L. & Kayser, O. Antileishmanial structure- activity relationships of synthetic phospholipids: *in vitro* and *in vivo* activities of selected derivatives. *Antimicrob. Agents Chemother.* **51,** 4525–4528 (2007).
63 Linnington, R. G. *et al.* Symplocamide A, a potent cytototoxin and chymotrypsin inhibitor from marine cyanobacterium. *Symploca sp. J. Nat. Prod.* **71,** 22–27 (2008).
64 Linnington, R. G., Gonzalez, J., Urena, L., Romero, L. I., Ortega-Barria, E. & Gerwick, W. H. Venturamides A and B: antimalarial constituents of the Panamanian marine cyanobacterium *Oscillatoria sp. J. Nat. Prod.* **70,** 397–401 (2007).
65 Mcphail, K. L. *et al.* Antimalarial linear lipopeptides from panamanian strain of marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.* **70,** 984–988 (2007).
66 Wright, A. D., Papendrof, O. & Koing, G. M. Ambigol C and 2, 4 dichlorobenzoic acid, natural products produced by terrestrial cyanobacterium *Fischerella ambigua*. *J. Nat. Prod.* **68,** 459–461 (2005).
67 Barbaraus, D., Kaiser, M., Brun, R. & Gademann, K. Potent and selective antiplasmodial activity of the cyanobacterial alkaloid nostocarboline and its dimers. *Bioorg. Med. Chem. Lett.* **18,** 4413–4415 (2008).
68 Portmann, C. *et al.* Isolation of aerucyclamides C and D and structure revision of microcyclamide 7806A, heterocyclic ribosomal peptides from *Microcystis aeruginosa PCC 7806* and their antiparasite evaluation. *J. Nat. Prod.* **71,** 1891–1896 (2008).
69 Clark, B. R. *et al.* Natural products chemistry and taxonomy of the marine cyanobacterium. *Blennothrix cantharidosmum*. *J. Nat. Prod.* **71,** 1530–1537 (2008).
70 Bister, B. *et al.* Cyanopeptolin 963A, a chymotrypsin inhibitor of *Microcystis PCC 7806*. *J. Nat. Prod.* **67,** 1755–1757 (2004).
71 Elert, E., Oberer, L., Merkel, P., Huhn, T. & Blom, J. F. Cyanopeptolin 954, a chlorine-containing chymotrypsin inhibitor of Microcystis aeruginosa NIVA Cya 43. *J. Nat. Prod.* **68,** 1324–1327 (2005).
72 Taori, K., Matthew, S., Rocca, J. R., Paul, V. J. & Luesch, H. Lyngbyastatins 5–7, potent elastase inhibitor from Floridian marine cyanobacteria. *Lyngbya sp. J. Nat. Prod.* **70,** 1593–1600 (2007).
73 Matthew, S., Ross, C., Rocca, J. R., Paul, V. J. & Luesch, H. Lyngbyastatin 4, a Dolastatin 13 Analogue with elastase and chymotrypsin inhibitory Activity from the marine cyanobacterium *Lyngbya confervoides*. *J. Nat. Prod.* **70,** 124–127 (2007).
74 Baumann, H. I. *et al.* Planktocyclin, a cyclooctapeptide protease inhibitor produced by the freshwater cyanobacterium *Planktothrix rubescens*. *J. Nat. Prod.* **70,** 1611–1615 (2007).
75 Taori, K., Paul, V. J. & Leusch, H. Kempopeptins A & B, protease inhibitors with different selectivity profiles from a marine cyanobacterium *Lyngbya sp. J. Nat. Prod.* **71,** 1625–1629 (2008).
76 Hayashi, O., Katoh, T. & Okuwaki, Y. Enhancement of antibody production in mice by dietary *Spirulina platensis*. *J. Nutr. Sci. Vitaminol.* **40,** 431–441 (1994).
77 Qureshi, M. & Ali, R. Spirulina platensis exposure enhances macrophage phagocytic function in cats. *Immunopharmacol. Immunotoxicol.* **18,** 457–463 (1996).
78 News, UC Davis School of Medicine and Medical Center, Dec. 2000.
79 Khan, Z., Bhadouria, P. & Bisen, P. S. Nutritional and therapeutic potential of Spirulina. *Curr. Pharm. Biotechnol.* **6,** 373–379 (2005).
80 Park, H. J. *et al.* A randomized double-blind, placebo-controlled study to establish the effects of spirulina in elderly Koreans. *Ann. Nutr. Metabol.* **52,** 322–328 (2008).
81 Mundt, S., Kreitlow, S., Novotony, A. & Effmert, U. Biochemical and pharmacological investigations of selected cyanobacteria. *Int. J. Environ. Health.* **203,** 327–334 (2001).
82 Benninga, J., Rock, K. L. & Goldberg, A. L. Interferon-γ can stimulate post-proteasomal trimming of the N terminus of an antigenic peptide by inducing leucine aminopeptidase. *J. Biol. Chem.* **273,** 18734–18742 (1998).
83 Shen, P. P. *et al.* Effects of cyanobacteria bloom extract on some parameters of immune function in mice. *Toxicol Lett.* **143,** 27–36 (2003).
84 Shi, Q. *et al.* Expression modulation of multiple cytokines *in vivo* by cyanobacterial blooms extract from Taihu Lake, China. *Toxicon* **44,** 871–879 (2004).