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Research review paper

Harmful and beneficial properties of cyanotoxins: Two sides of the same coin

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ABSTRACT

Cyanotoxins are by definition "harmful agents" produced by cyanobacteria. Their toxicity has been extensively studied and reviewed over the years. Cyanotoxins have been commonly classified, based on their poisonous effects on mammals, into three main classes, neurotoxins, hepatotoxins and dermatotoxins, and, considering their chemical features, mainly identified as peptides, alkaloids and lipopolysaccharides. Here we propose a broader subdivision of cyanotoxins into eight distinct classes, taking into account their molecular structures, biosynthesis and modes of action: alkaloids, non-ribosomal peptides, polyketides, non-protein amino acids, indole alkaloids, organophosphates, lipopeptides and lipoglycans. For each class, the structures and primary mechanisms of toxicity of the main representative cyanotoxins are reported. Despite their powerful biological activities, only recently scientists have considered the biotechnological potential of cyanotoxins, and their applications both in medical and in industrial settings, even if only a few of these have reached the biotech market. In this perspective, we discuss the potential uses of cyanotoxins as anticancer, antimicrobial, and biocidal agents, as common applications for cytotoxic compounds. Furthermore, taking into account their mechanisms of action, we describe peculiar potential bioactivities for several cyanotoxin classes, such as local anaesthetics, antithrombotics, neuroplasticity promoters, immunomodulating and antifouling agents. In this review, we aim to stimulate research on the potential beneficial roles of cyanotoxins, which require interdisciplinary cooperation to facilitate the discovery of innovative biotechnologies.

1. Introduction

1.1. Biology and ecology of cyanobacteria

Cyanobacteria, otherwise known as blue-green algae, are a wide and heterogeneous group of prokaryotic organisms described as the most ancient photoautotrophs, with the broadest geographical distribution on Earth (Gaysina et al., 2019). Cyanobacteria reached this success through various trajectories of adaptive evolution, which enabled them to live both in aquatic and terrestrial ecosystems and to survive, under a wide range of environmental stresses, in extreme environments like hot springs, polar regions and desert soils (Billi et al., 2017; Kvíderová et al., 2019; Alcorta et al., 2020).

Cyanobacteria metabolic plasticity is due, to a large extent, to their ancient origin in an anoxic biosphere of the Early Earth and their initial capability of growing in a scarcity of fundamental nutrients (Schirrmeister et al., 2016; Reinhard et al., 2017; Zerkle et al., 2017). Later, with the atmospheric changes occurred during the Great Oxidation Event (GOE), early cyanobacteria developed specific metabolic pathways to adapt to variable conditions of light, temperature and macro- and micro-nutrients availability (de Marsac and Houmard, 1993). These peculiar pathways are not included in their primary metabolism, which is mainly involved in the regulation of essential functions such as growth, development and reproduction. Conversely, secondary metabolism mainly refers to those mechanisms necessary for cyanobacteria survival, involved, for example, in UV-light protection, prevention of oxidative stress (Sinha and Häder, 2008; Latifi et al., 2009), or competition for light and nutrients with other sympatric species (Mur et al., 1999).

In aquatic environments, cyanobacteria may be found free-living in

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the water column or associated to other micro- and macroorganisms, forming for example benthic mats, whereas terrestrial cyanobacteria are often associated to soil crusts, which mainly colonize dryland areas (Büdel et al., 2016; Huisman et al., 2018; Vidal et al., 2021). This capability of establishing ecological relationships (including symbiosis) with a range of organisms allowed cyanobacteria to evolve different mechanisms to promote maximum advantages from and for the host (Rai et al., 2002).

Most of these forms of life are to be considered as complex communities, where several taxa cohabit in a dynamic equilibrium. Often the winning competition for nutrients, oxygen and light, facilitated by the release of secondary metabolites, make cyanobacteria dominant in aquatic communities. Such metabolites may be defined as "allelochemicals" (from the Greek ἀλλήλων, allēlōn, meaning "mutual", "reciprocal"), a term which indicates compounds involved in the competitive interactions between organisms; and the biological phenomenon that defines this behaviour is referred to as "allelopathy" (McCoy et al., 2022).

The first definition of allelopathy was given by Hans Molisch in 1937, a Czech-Austrian botanist who observed how early-ripening fruits can induce maturation of late-ripening fruits when stored together. He first used this term to define the phenomenon of mutual influence between one plant and another (Molisch, 1937). The concept was further adopted, in a broader vision, to define "any direct or indirect harmful or beneficial effect by one organism on a another through production of chemical compounds that escape into the environment" (Rice, 1984). Consequently, the active release of allelochemicals can exert a direct or indirect effect on the surrounding biota, influencing their growth, physiology and behaviour (Leflaive and Ten-Hage, 2007; Leão et al., 2009).

Cyanobacteria are often found in fast-growing aquatic biomasses, compared to other photoautotrophs, releasing a set of allelochemical weapons that dominate the space in highly competitive environments. This is a phenomenon known under the name of "harmful algal blooms" (HAB) (Dias et al., 2017), which may be harmful for humans, animals, plants and other cohabiting organisms. Generally, various environmental factors concur to such cyanobacterial proliferation, such as high nitrogen and phosphorous content, light intensity and temperature trends (Paerl, 2017). This explains why climate changes, along with water pollution, may contribute to the exacerbation of eutrophication and, consequently, of cyanobacteria blooms (Huisman et al., 2018; Nazari-Sharabian et al., 2018; Gobler, 2020). In this perspective, the multitude of toxic compounds produced by cyanobacteria are referred to as cyanotoxins, which have been studied both from a toxicological and a biological perspective (Chorus and Bartam, 1999).

1.2. Toxicity of cyanobacteria

The first documentation of cyanobacterial toxicity was reported by George Francis (1878), a South Australian chemist who conducted pioneering studies on the quality of drinking water. He reported episodes of animal poisonings occurring as a consequence of drinking from Lake Alexandrina in South Australia, with initial symptoms such as unconsciousness and stupefaction, followed by convulsions, spasms and finally death. The organism responsible was identified by Francis as *Nodularia spumigena*, a bloom-forming diazotrophic green-blue alga, subsequently reported as producer of the toxin nodularin (Rinehart et al., 1988).

Later, many other species were discovered and identified as toxin-producing cyanobacteria, such as *Microcystis spp.*, *Anabaena spp.*, *Oscillatoria spp.*, *Nostoc spp.*, and *Lyngbya* spp. among others (Carmichael, 2001; Cheung et al., 2013; Buratti et al., 2017; Metcalf and Codd, 2020).

Over the years, most research has focused on understanding the mechanisms underpinning the physiological regulation and the biological functions of cyanotoxins (Sivonen and Jones, 1999; Humbert, 2009; Pearson et al., 2016; Sanseverino et al., 2017). They are commonly classified as secondary metabolites, but their role in growth, development and reproduction is still not fully clarified (Carmichael, 1992; Orr and Jones, 1998; Yunes, 2019). It has been hypothesized that they have evolved in peculiar environmental conditions to override other species in very competitive habitats or, in other contexts, it has been assumed that they evolved to aid general physiological functions, such as maintenance of homeostasis, iron scavenging or cell-cell signalling (Holland and Kinnear, 2013).

However, to date, the original ecological role of cyanotoxins is still a matter of debate. Conversely, their toxicological role has been thoroughly defined. Numerous research articles and systematic reviews have been published reporting cyanobacteria as dangerous toxin producers, describing cyanotoxins harmful effects, or focusing on the development of strategies for their monitoring and removal from various environments (Wang et al., 2021; Abdallah et al., 2021; Serrà et al., 2021; Miglione et al., 2021; Lei et al., 2022).

What is not yet widely accepted is the concept that most of the toxins produced by cyanobacteria have powerful biological activities that may be worth exploiting in agriculture, pharmacology, cosmetology, and many other industrial fields.

In this review, we offer a renewed vision of cyanotoxins, for years pointed out only as harmful agents. Here, several classes of cyanotoxins known so far have been reviewed regarding their origin, isolation, chemical structures, and mechanisms of toxicity on mammals. In addition, their potential biotechnological applications, both for industrial and medical scopes, are discussed.

2. Cyanotoxins

Over the years, cyanotoxins have been grouped into a few different classes on the basis of their toxicity (neurotoxins, dermatotoxins, hepatotoxins, cytotoxins) (Humbert, 2009; Metcalf and Codd, 2014; Sanseverino et al., 2017; Machado et al., 2018) or based on their chemical nature (alkaloids, cyclic peptides, lipopolysaccharides) (Chorus and Bartam, 1999; van Apeldoorn et al., 2007). In this review, we provide a broader subdivision of cyanotoxins into eight main classes (Fig. 1), considering their molecular structures, biosynthesis and modes of action.

2.1. Alkaloids

Alkaloids represent the most abundant class of defensive secondary metabolites. More than 27,000 alkaloids are currently known in nature (Parthasarathy et al., 2021) endowed with a wide range of applications in pharmaceutical, medical, and agricultural settings (Aniszewski, 2015). Depending on their molecular structure and origin, they are in turn classified into true alkaloids, protoalkaloids and pseudoalkaloids but, in general, they all possess a cyclic backbone that contains nitrogen in a negative oxidation state (Pelletier, 1983).

Alkaloid cyanotoxins are mainly represented by anatoxins (ATXs) and saxitoxins (STXs), also known as neurotoxins, because of their primary effects related to the nerves and the muscles they control (Christensen and Khan, 2020), and cylindrospermopsins (CYNs), identified more in general as cytotoxins, due to their multiple toxicity on liver, kidney and nervous system, which is probably a direct consequence of their wide distribution through body tissues and organs (Mathe et al., 2017).

As for their chemical nature, anatoxin-a (Fig. 2) is an asymmetrical bicyclic secondary amine (Devlin et al., 1977), and so as its analogue, homoanatoxin-a, which differs from anatoxin-a only for an additional methyl group on the carbon 11 (Wonnacott et al., 1992). Whereas saxitoxin, also known as paralytic shellfish poisoning (PSP) toxin, is constituted by a tricyclic perhydropurine backbone, and cylindrospermopsin consists of a polycyclic uracil derivative containing guanidin and sulfate groups (Fig. 2).

Anatoxin-a and homoanatoxin-a act as potent agonists of the

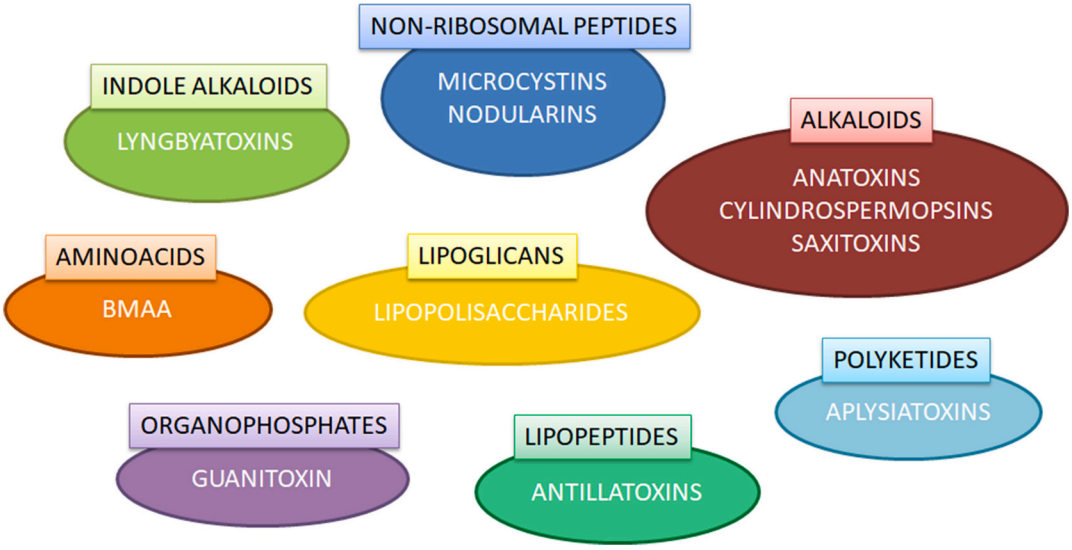

Fig. 1. Cyanotoxins subdivision in classes with main representatives.

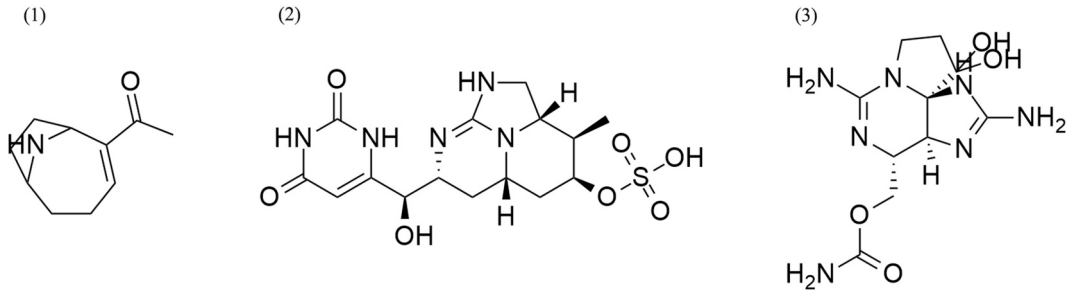

Fig. 2. Chemical structures of alkaloid cyanotoxins: 1) anatoxin-a, 2) cylindrospermopsin, 3) saxitoxin.

muscular and neuronal nicotinic acetylcholine receptor (nAChR). Their binding to this ionotropic receptor leads to the opening of the channel and thus to the flow of cations (K^+, Na^+, Ca^{2+}), provoking the depolarization of the cell membrane (Bruno et al., 2016; WHO, 2020a). Saxitoxins, instead, behave as blockers of the voltage-gated sodium channels (VGSCs) in neuronal cells, thus inhibiting propagation of an action potential along neuronal axons, or rather reducing or eliminating the transmission of a nerve impulse (Evans, 1969; Thottumkara et al., 2014; WHO, 2020b).

Despite their different molecular properties and modes of action, ATXs and SXTs share the same acute neuroxicity when ingested, causing similar symptoms of paralysis and respiratory failure.

On the other hand, cylindrospermopsins show a wide range of toxic effects. Even if the liver is the main target, other organs, such as intestinal tract, kidney, spleen, and thymus may be affected. Although not clearly understood, more than one mode of action could be involved in cylindrospermopsins toxicity (Evans and Murphy, 2011; WHO, 2020c). Inhibition of protein synthesis seems to be the primary mechanism of their activity, but some others have also been reported, such as inhibition of glutathione synthesis, DNA damage, induction of oxidative stress (Froscio et al., 2003; Humpage et al., 2005).

2.2. *Non-ribosomal peptides*

Non-ribosomal peptides (NRPs) are synthetized by a specific non-ribosomal peptide synthetase (NRPS) enzyme complex in an RNA-independent synthetic pathway (Neilan et al., 1999). NRPs are small molecular weight cyclic hepta- and penta- peptides (Carmichael et al., 1988), among which microcystins (MCYSTs) and nodularins (NODLNs) are the class leaders.

MCYSTs are the most abundant class of cyanobacterial toxins, with almost 300 congeners already identified (Bouaïcha et al., 2019). Their general chemical structure is a cyclo-(D-Ala-X-D-Masp-Z-Adda-D-Glu-Mdha), where X and Z are variable L-amino acids. Whereas NODLNs share the same cyclic peptide structure but lack the two sites for the variable L-amino acids (Rinehart et al., 1988). As an example, the structures of microcystin-LR (L-Leu and L-Arg as X and Z) and nodularin are reported in Fig. 3.

MCYSTs and NODLNs act as inhibitors of the serine/threonine protein phosphatase families PP1 and PP2A, leading to hyperphosphorylation of functional and cytoskeletal proteins, followed by cell process alterations (cell-cell adhesion, actin filaments structuring, MAPKs signalling) and finally apoptosis (Yoshizawa et al., 1990). Moreover, they are pro-oxidants with the potency to induce cell damaging oxidative stress through generation of reactive oxygen species (ROS), with subsequent genotoxic effects such as DNA fragmentation, chromosomal aberrations or base substitution mutations (Bouaïcha and Maatouk, 2004). Their high hepatotoxicity is not strictly related to the mechanism of action, but rather to their distribution to the tissues (WHO, 2020d). In fact, due to their relatively large and bulky structures, MCYSTs and NODLNs are unable to cross cell membranes by passive diffusion, and require active uptake by cells, by means of organic anion transporters polypeptides (OATPs), in particular OATP1B1 and OATP1B3 which, in healthy humans, are found to be largely expressed in liver tissue (Fischer et al., 2005; Hagenbuch and Gui, 2008).

Peptides other than MCYSTs and NODLNs have been poorly explored, and information is lacking on their ecological role, toxicity, and impact on human health. Among these, anabaenopeptins (APs), a

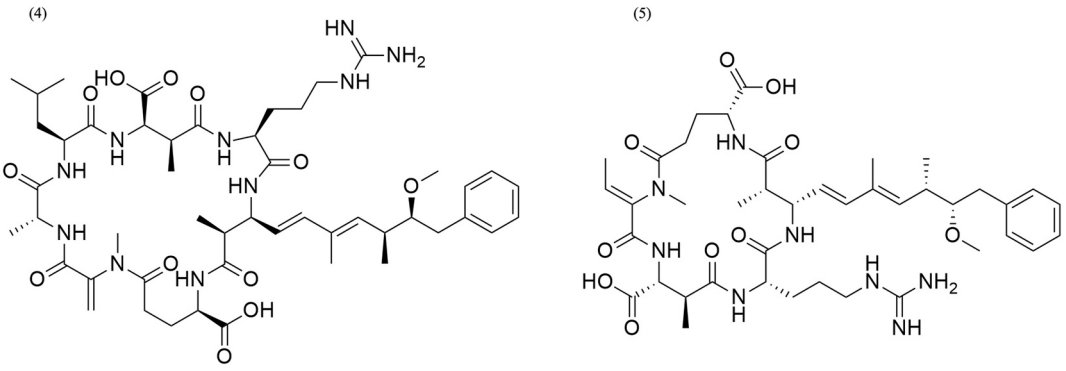

Fig. 3. Chemical structures of peptide cyanotoxins: 4) microcystins-LR, 5) nodularin.

family of cyclic peptides composed by six amino-acid residues (see anabaenopeptin B structure in Fig. 4), have lately received increasing attention (Spoof et al., 2015; Monteiro et al., 2021). APs are well known for their inhibiting activity of carboxypeptidases, phosphatases and proteases (Itou et al., 1999; Sano et al., 2001; Gesner-Apter and Carmeli, 2009), enzymes generally involved in the regulation of several vital physiological and metabolic processes. APs toxicological effects have only been reported in the model nematode *Caenorhabditis elegans* (Lenz et al., 2019), but whether these observed toxic effects are related to the known enzyme inhibition properties of these cyanopeptides remains to be elucidated.

Recently, several new depsipeptides (peptides with an ester linkage) from cyanobacteria have been characterised. Belonging to this class, lyngbyabellins (LYBs) are cyclic or linear cytotoxic depsipeptides, characterized by two thiazole rings and a peculiar gem-dichloro group (CCl_2) (see lyngbyabellin A structure in Fig. 4) (Choi et al., 2012). LYBs act as actin-disassembling agents, therefore as disrupters of the cellular microfilament network. In particular, they were reported to inhibit cytokinesis inducing alterations in cell morphology (Luesch et al., 2000a; Han et al., 2005).

2.3. Polyketides

Generally, polyketides represent a huge class of natural products sharing a common mechanism of ribosome-independent biosynthesis involving a class of enzymes called polyketide synthases (PKSs). Despite aplysiatoxins (APTXs) represent a distinct polyketide class of toxins isolated from several cyanobacterial species, their biosynthetic pathway has not been elucidated so far. It was proposed to be related to the formation of a pyran ring system, with high level of methylation, and the combination of two polyketide units to form a macrolactone (see aplysiatoxin structure in Fig. 5) (Tidgewell et al., 2010). Aplysiatoxin and debromoaplysiatoxin are classified as dermatotoxins, since they induce potent skin irritation through the activation of protein kinase C (PKC), thus causing rashes and skin blisters (Osborne et al., 2001). Moreover, since PKC have important roles in cell-cycle regulation, its excessive

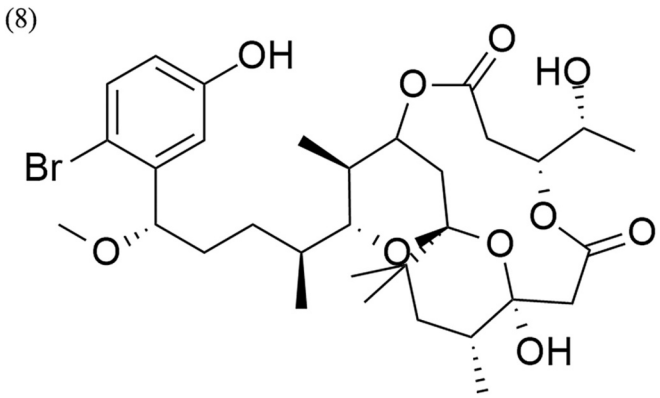

Fig. 5. Chemical structures of polyketide cyanotoxin: 8) aplysiatoxin.

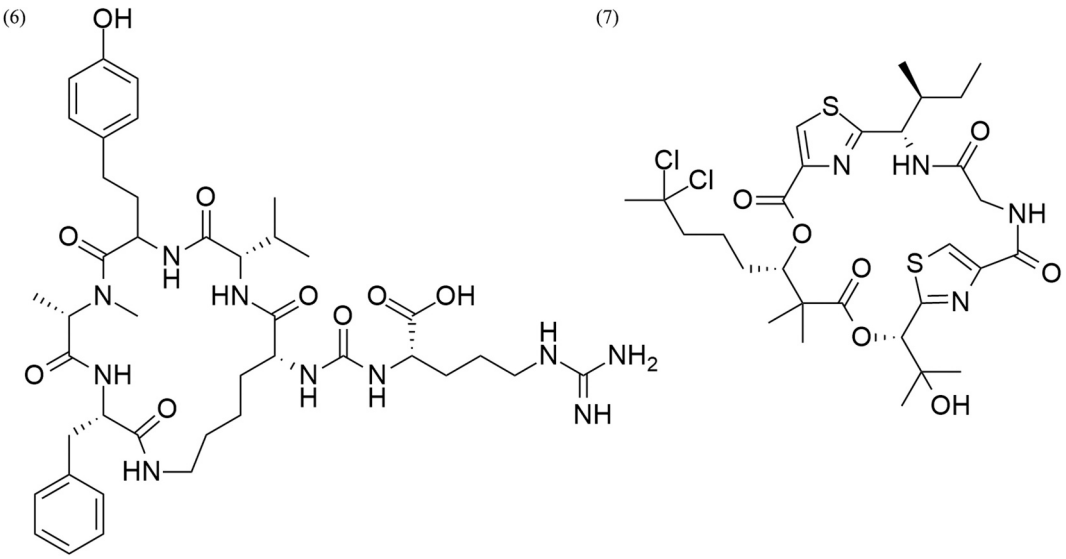

Fig. 4. Chemical structures of peptide cyanotoxins: 6) anabaenopeptin B, 7) lyngbyabellin A.

activation can lead to carcinogenesis. In fact, APTXs are also reported as strong tumor-promoting factors (Horowitz et al., 1983).

2.4. Non-protein amino acids

β-N-methylamino-L-alanine (BMAA) and its naturally occurring isomers belong to the class of non-protein amino acidic (NPAA) toxins. They are non-canonical amino acids. Indeed, although they contain an amino- and a carboxyl- group, they do not take part in protein synthesis. BMAA is actually an alanine, but with a methylamino group on the side chain, whereas its two most studied constitutional isomers, 2,4-diaminobutyric acid (DAB), N-(2-aminoethyl) glycine (AEG), basically exhibit the same molecular structure, but different connectivity (Fig. 6) (Jiang et al., 2012). BMAA was first identified as the causative agent of amyotrophic lateral sclerosis/Parkinsonian-dementia complex (ALS/PDC) (Vega and Bell, 1967; Spencer et al., 1987; Cox et al., 2003), a neurodegenerative disease frequently occurred among the Chamorro, indigenous population of the small Pacific Island of Guam, in the 1950s (Arnold et al., 1953). BMAA neurotoxic activity involves different targets through several mechanisms which add up to favour the development of neurodegenerative processes. Once in the human body, BMAA tends, in presence of bicarbonate ions (HCO_3^-), to form carbamate adducts structurally similar to glutamate, therefore acting as glutamate receptor agonists (Weiss et al., 1989; Rao et al., 2006). The activation of glutamate receptors, both ionotropic (iGluRs) and metabotropic (mGluRs), induces a significant increase in intracellular Ca^{2+}, which promotes reactive oxygen species (ROS) generation, degradation of proteins, lipids, and nucleic acids, loss of neuronal function and cell death (Chiu et al., 2012). This neurotoxic cascade is defined excitotoxicity (Armada-Moreira et al., 2020), which is the most accredited BMAA mechanism of action, but not the only one. In fact, BMAA was also reported to accumulate into brain tissues by associating with host proteins, as a consequence of a misincorporation of BMAA into the primary structure of proteins, in the place of L-serine, or more likely by a direct BMAA-protein interaction, thus triggering protein misfunction, misfolding and/or aggregation (Glover et al., 2014; van Onselen et al., 2015). Although the molecular mechanisms of neurotoxicity remain to be elucidated, its neurodegenerative effects are well ascertained, even if the association between BMAA exposure and susceptibility to neurodegenerative diseases is still a current matter of study.

2.5. Indole alkaloids

Indole alkaloids are a class of alkaloids structurally consisting of a highly functionalized polycyclic ring system based on an indole core (Walton and Berry, 2016). Lyngbyatoxins (LTXs) are the main representatives of indole alkaloid toxins, constituted by an indolactam and a monoterpenoid moiety (Cardellina 2nd et al., 1979). Lyngbyatoxin-a (Fig. 7), identified as the causative agent of seaweed dermatitis from the Hawaiian *Lyngbya majuscule*, is a highly inflammatory and vesicatory skin irritant, therefore defined as dermatotoxin. The mechanism of action, through which LTXs exert their toxicity, is similar to that of aplysiatoxins, which involves activation of PKC. LTXs was also reported to have a tumor-promoting activity comparable to that of 12-O-tetradecanoylphorbol 13-acetate (TPA) in vivo (Fujiki et al., 1984).

Newly discovered, hapalindoles (HIs) include a diversified group of indole alkaloids derivatives, with tetra- and tricyclic core ring systems (see hapalindole H structure in Fig. 7). Given the evidence on their sodium channel-modulating activity, hapalindoles can be defined as full-fledged neurotoxins. Their toxicity is reported to be similar to that of neo-saxitoxin, and may be related to ion-dependent disturbances of excitable membranes, with a subsequent blocking of axonal conduction, and interruption of the propagation of nerve impulses (Cagide et al., 2014). They are also precursors for the so-called hapalindole-type alkaloids, and namely hapalindolinones, ambiguines, fischambiguines, fischerindoles and welwitindolinones, characterized by very complex structures and interesting biological activities (Hohlman and Sherman, 2021).

2.6. Organophosphates

The only member of this class of cyanotoxins is anatoxin-a(s) (ATX-a (s)), a guanidinium methyl phosphate ester (Fig. 8) (Matsunaga et al., 1989). Initially known as a neurotoxic alkaloid, it has been grouped with anatoxins. Recently, given the substantial differences in the chemical structure, mechanism of action and biosynthesis, it has been renamed guanitoxin (GNT) (Fiore et al., 2020), to emphasize its distinctive guanidine organophosphate chemical structure. GNT acts as an irreversible inhibitor of acetylcholinesterase (AChE), by a similar mechanism as the organophosphates and carbamate insecticides (Mahmood and Carmichael, 1986, 1987). The enzymatic inhibition occurs through a covalent bond between the serine residue of acetylcholinesterase and the phosphate group of the toxin, which leads to inhibition of acetylcholine (ACh) recycling and its subsequent accumulation. As a consequence, ACh remains available and binds membrane receptors of the peripheral nervous system, resulting in continuous muscle stimulation, thus leading to convulsions, muscle fatigue, and respiratory arrest (Patočka et al., 2011).

2.7. Lipopeptides

Lipopeptides are molecules composed by peptide and fatty acid chains bound together. Those produced by cyanobacteria include cytotoxic lipopeptides with linear and cyclic structures (intracyclic or exocyclic lipopeptides) (Fewer et al., 2021). Anabaenolysins (ABLs) are cytotoxic cyclic lipopeptides (see anabaenolysin A structure in Fig. 9), consisting of a four-membered peptide ring, composed by two proteinogenic amino acids (glycine, glycine) and the unusual 2-(3-amino-5-oxotetrahydrofuran-2-yl)-2-hydroxyacetic acid moiety, and of a long unsaturated C18 β-amino fatty acid with a conjugated triene structure (Jokela et al., 2012). Their amphipathic structure is the key factor of their cytotoxicity. In fact, they act as biodetergents, by penetrating cholesterol-containing cell membranes, inducing membrane destabilization and cell lysis (Oftedal et al., 2012).

Another class of lipopeptide toxins are Antillatoxins (ANTXs), classified as neurotoxins because of their action on neuronal VGSCs (Aráoz et al., 2010). ANTXs possess a cyclic tripeptide backbone bonded together with a highly methylated lipid portion (see antillatoxin B structure in Fig. 9). Unlike other neurotoxins, they act as activators of neuronal VGSCs. The ensuing increase of intracellular Na^+ provoke overstimulation of N-methyl-D-aspartate receptor (NMDARs). The abnormal activation of this class of ionotropic glutamate receptors is

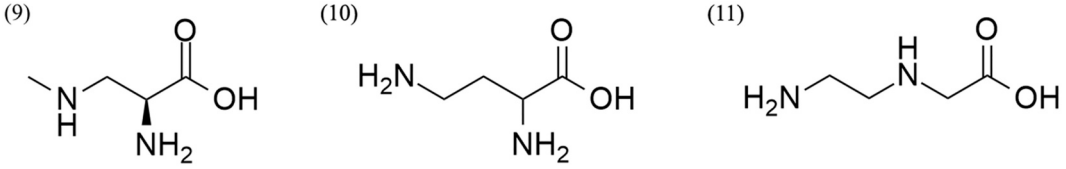

Fig. 6. Chemical structures of NPAA cyanotoxins: 9) BMAA, 10) DAB, 11) AEG.

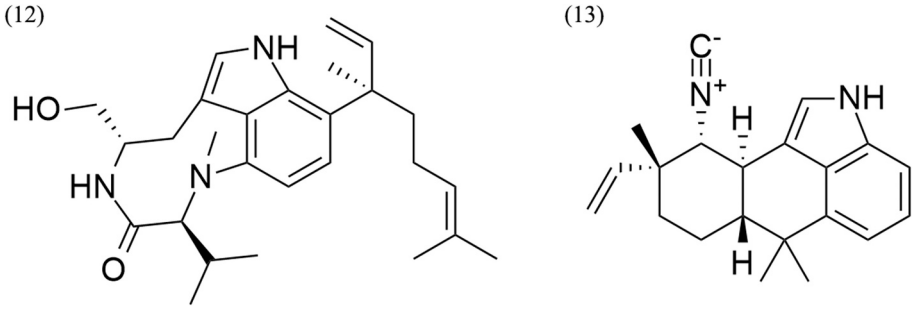

Fig. 7. Chemical structures of indole alkaloid cyanotoxins: 12) lyngbyatoxin A, 13) hapalindole H.

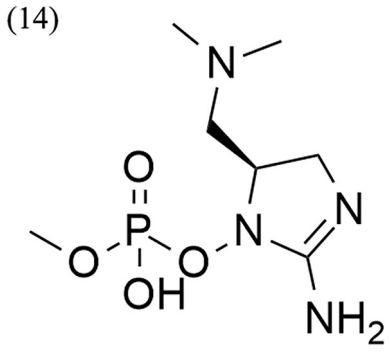

Fig. 8. Chemical structures of organophosphate cyanotoxin: 14) guanitoxin.

involved in triggering excitotoxicity, provoking swelling of neuronal somata, thinning of neuritis, and blebbing of the neurite membrane (Berman et al., 1999).

2.8. Lipoglycans

Belonging to the lipoglycans chemical class, lipopolysaccharides (LPSs) represent the most common class of toxins. LPS is a structural component of the outer cell membrane of gram-negative bacteria (including cyanobacteria), isolated from most cyanobacterial species. Compared to all other classes of exotoxins, which are secreted or released into the surrounding environment, LPSs are generally classified as endotoxins.

Structurally, LPS is a complex polymer composed of three main domains, the lipid A, a polysaccharide core and the O-antigen. Those of cyanobacteria exhibit slight structural differences compared to proteobacterial LPSs, such as the lack of phosphates in the lipid A region and of 3-deoxy-D-*manno*-oct-2-ulosonic acid (Kdo) and heptose residues in the polysaccharide portion, which, alternatively, is mainly composed by neutral sugars (Snyder et al., 2009; Fujii et al., 2012). These endotoxins generally elicit a strong immune response when in contact with the human body, inducing effects ranging from gastro-intestinal to respiratory illnesses, from pyrexia to septic shock (Stewart et al., 2006). The mechanism by which proteobacterial LPSs induce inflammation is via Toll-like receptor 4 (TLR-4), whose activation induces significant release of pro-inflammatory cytokines, such as TNF-α, IL-8, IL-6 (Mazgaeen and Gurung, 2020).

This is presumably what also happens in the case of cyanobacterial endotoxins. However, interactions of cyanobacterial LPSs with the immune system are still scarcely understood and the role of structurally different cyanobacterial lipid A in TLR4 signalling is under investigation. It is indeed reported that some LPSs from *Oscillatoria* and *Synechococcus* species (Fig. 10) possess a tetra-acylated lipid A portion (Snyder et al., 2009; Carillo et al., 2014; Durai et al., 2015). This peculiar structure allows LPS recognition by the MD2-TLR4 complex but prevents the dimerization and therefore the activation of the receptor, inducing an antagonist-like effect (Matsuura, 2013).

3. Cyanotoxins applications for biotechnological purposes

Several reviews have been published so far, summarizing the cyanotoxins harmful effects on human health, animals, plants or other organisms with which they can come into contact in the environment (Carmichael, 1992; van Apeldoorn et al., 2007; Funari and Testai, 2008; Ferrão-Filho Ada and Kozlowsky-Suzuki, 2011; Drobac et al., 2013; Buratti et al., 2017; Metcalf and Codd, 2020; Plaas and Paerl, 2021), but less information has been reported about their biotechnological potential uses. Recently, Demay et al. (2019) described the beneficial activities of natural products from cyanobacteria, including ten different chemical classes of molecules, without explicitly distinguishing between cyanotoxins and other non-toxic metabolites. A similar approach was adopted by Khalifa et al. (2021), and Abed et al. (2009) about ten years earlier, who have reported the biotechnological applications of cyanobacteria as cell factories and their bioactive compounds, only mentioning a few classes of cyanotoxins. In order to highlight the scarce attention of the scientific community on the potential biotechnological applications of cyanotoxins, we conducted a bibliometric analysis of papers considering cyanotoxins as the main topic, published during the last 10 years, reporting the twenty most frequent research fields of interest (Fig. 11).

The literature available on cyanotoxins covers more than 100 research areas, even though most of the papers remain in the field of environmental sciences and ecology, whereas just over 10% are in the field of biotechnology and applied microbiology. Here, we focused only

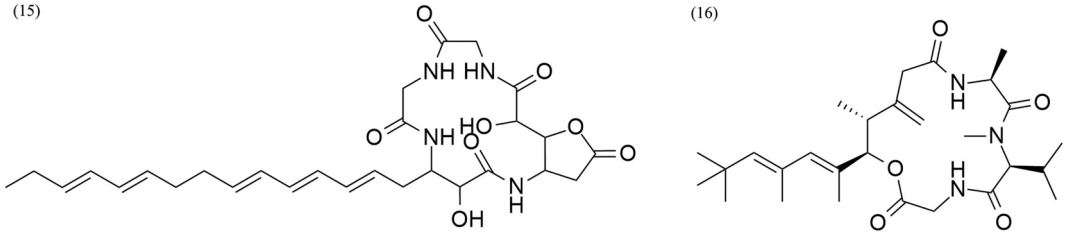

Fig. 9. Chemical structures of lipopeptide cyanotoxins: 15) anabaenolysin A, 16) antillatoxin B.

(17)

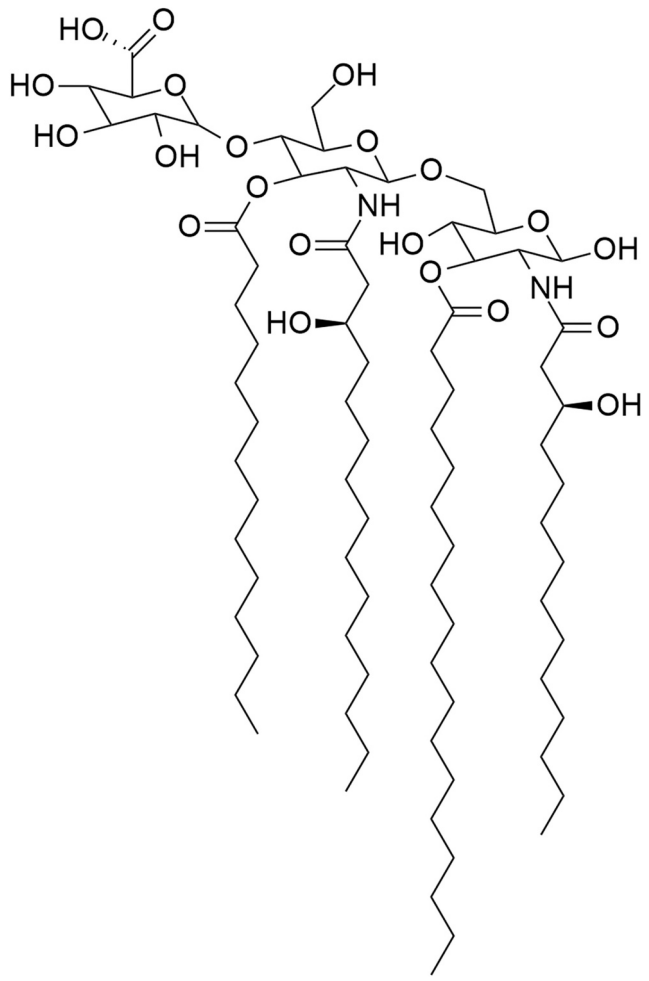

Fig. 10. Chemical structures of lipid A portion from *Synechococcus* LPS.

on cyanobacteria-derived compounds hitherto labelled as "toxic", but highlighting their beneficial effects in clinical settings and their potential biotechnological applications. In detail, several cyanotoxins with anticancer, antimicrobial, biocidal and other biomedical applications were searched in the literature, organized according to their mechanism and bioactivity, as reported in Table 1.

3.1. Anticancer

The first evidence that cyanobacteria were able to produce compounds endowed with anticancer activity was provided by Mynderse et al. (1977). They first examined the antileukemia activity of several species of the *Oscillatoriaceae* family, testing their chloroform extracts on the P-388 murine leukemia cell line. The purification of the extracts and the characterization of the obtained active fractions led them to isolate the P-388 active compound, i.e., debromoaplysiatoxin (Fig. 12), the same compound allegedly responsible for dermal toxicity of *Lyngbya* species (Grauer and Arnold Jr, 1961), later confirmed by Solomon and Stoughton (1978). From this study as a starting point, Kashiwagi et al. (1980) examined the antineoplastic effects of crude extracts obtained from 107 specimens of marine algae collected off the Pacific Islands, reporting excellent anti-proliferative activities at relatively low dosages, with no evidence of toxicity, on murine leukaemia and Ehrlich-Lettre ascites carcinoma cell lines. These studies have paved the way towards a more conscious search for molecules with anticancer activity from cyanobacteria. Indeed, shortly thereafter Patterson et al. (1991) started a large-scale screening program aimed at the isolation of antineoplastic agents from laboratory-cultured blue-green algae, finally establishing how precious cyanobacteria could be as a source of anticancer drugs.

One of the first identified anticancer cyanotoxins was curacin A (Fig. 12), a unique thiazoline-containing lipopeptide isolated from a marine Caribbean *Lyngbya majuscule* strain, endowed with an antimitotic activity against renal, colon and breast cancer cell lines (Gerwick et al., 1994; Wipf et al., 2004). It was found to inhibit microtubule assembly with an IC_{50} value of 0.70 μM, selectively binding to the colchicine site of tubulin, and to induce cell cycle arrest at the G2/M phase (Gerwick et al., 1994; Verdier-Pinard et al., 1998). Due to its therapeutic potential, curacin A underwent early clinical trial phases but, unfortunately, it was ruled out because of its high lipophilicity. However, over the years, several attempts were made to identify curacin A congeners or synthetic analogues with improved water solubility and chemical stability, suitable for further drug development as anticancer agents (Yoo and Gerwick, 1995; Márquez et al., 1998; Verdier-Pinard et al., 1998; Wipf et al., 2002, 2004; Singh et al., 2008).

At the same time, another antimitotic agent was isolated from cyanobacteria of the genus *Nostoc*, cryptophycin 1 (Fig. 12), a cyclic peptide cyanotoxin with a potent anti-proliferative activity against various cancer cell lines, with IC_{50} values lower than 50 pM (Lieberman et al., 2001). Cryptophycin 1 interferes with microtubule assembly processes with a mechanism of action similar to that of Vinca alkaloids (Smith et al., 1994; Bai et al., 1996). In detail, it inhibits microtubule polymerization, causing tubulin to aggregate, and disassembles microtubules to linear polymers somewhat similar to the spiral-like structures produced by the Vinca alkaloids (Kerksiek et al., 1995). As a consequence, it hinders the formation of mitotic spindles during the cell cycle, thus leading to mitotic arrest (Mooberry et al., 1997). Due to its potential, the research of analogues of cryptophycin, either naturally isolated or chemically synthesized, has attracted the attention of the scientific community. Successfully, cryptophycin-52 proved to be an excellent synthetic candidate (Chen et al., 1998). In addition to the microtubule inhibition activity, it was shown to induce apoptosis by Bcl-2 phosphorylation in human H460 non-small-cell lung carcinoma (NSCLC) cell line (Lu et al., 2001). It entered a phase II clinical trial, but it failed to produce measurable responses and induced a significant level of neurotoxicity, and therefore was not approved for clinical use (Edelman et al., 2003). However, cryptophycins have stood out for their potential use in targeted therapeutic approaches and have been recently involved in the development of antibody-drug conjugates (ADCs) and small molecule-drug conjugates (SMDCs). These latter showed no toxicity, improved stability in plasma and high selective anticancer effects, demonstrating their potential for the targeted therapy of solid tumors (Verma et al., 2015; Borbély et al., 2019; Lai et al., 2020; Anselmi et al., 2021).

Apratoxins represent another class of anticancer cyclic peptide cyanotoxins lately discovered from *Lyngbya* sp. (Luesch et al., 2001a). Apratoxin A (Fig. 12) prevents the biogenesis of secretory and membrane proteins, inhibiting cotranslational translocation into the endoplasmic reticulum (ER), by direct blockade of the Sec61 protein translocation channel (Liu et al., 2009; Paatero et al., 2016). It showed cytotoxicity against human osteosarcoma, colorectal and cervix adenocarcinoma, and breast, ovarian endometrial and pancreatic cancer cell lines (IC_{50} 0.36–18.6 nM) (Luesch et al., 2001a; Luesch et al., 2006; Ma et al., 2006; Huang et al., 2016). Apratoxin D and E were also isolated from different *Lyngbya* species, showing a higher and a lower anticancer activity, respectively, compared to their congener apratoxin A, but neither of them was further investigated for anticancer drug development (Gutiérrez et al., 2008; Matthew et al., 2008). Instead, some synthetic analogues have proceeded into clinical trials. For instance, apratoxins S10 was proved to induce a clear reduction of cancer proliferation through down-regulation of multiple receptor tyrosine kinases (RTKs), such as the vascular endothelial growth factor receptors

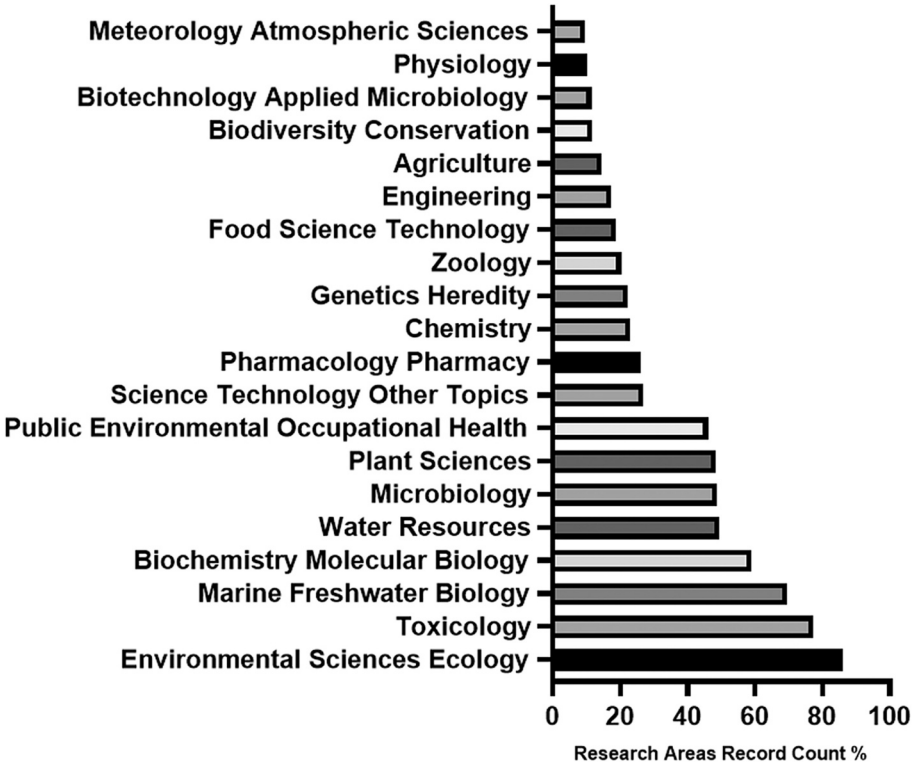

Fig. 11. Bibliometric analysis on "cyanotoxins" in terms of research areas using Web of Science database. The analysis included all research papers published from January 2013 to February 2022. The chart shows the 20 most frequent research fields.

(VEGFRs), therefore exerting a strong anti-angiogenic effect in addition to its potent anti-proliferative effect in highly vascularized cancer cell models (IC_{50} 0.83–3.35 nm) (Cai et al., 2017). Moreover, Apra S10 also inhibited tumor growth in a pancreatic patient-derived xenograft (PDX) preclinical model, thus resulting in a potentially good candidate for the treatment of pancreatic cancer (Cai et al., 2019).

Dolastatins are a family of linear peptide toxins endowed with a marked antimitotic activity, whose production has been initially attributed to the sea hare *Dolabella auricularia* (Bai et al., 1990; Pettit et al., 1993). Only twenty years later Hendrik Luesch and colleagues isolated dolastatin 10 (Fig. 12) from the marine cyanobacterium *Symploca* sp. VP642, then named symplostatin 1, suggesting that dolastatins isolated from the mollusc originated from a cyanobacterial diet (Luesch et al., 2001b). Dolastatin 10 and 15, and later their synthetic derivatives, Soblidotin (TZT-1027) and Cemadotin (LU103793), respectively, have shown very promising anticancer effects against different cancer cell lines, inducing depolymerization of microtubules in interphase cells with a subsequent formation of abnormal spindles and alteration of chromosome distribution in mitotic cells (Bai et al., 1990; Bai et al., 1992; de Arruda et al., 1995; Kobayashi et al., 1997; Poncet, 1999). Unsuccessfully, after entering clinical trials, neither natural nor synthetic dolastatins were found to be suitable for clinical use due to insufficient efficiency or induced peripheral neuropathy and neutropenia in treated patients (Luesch et al., 2002; Gao et al., 2021). To overcome the onset of these side effects, chemical researchers started to couple monoclonal antibodies with auristatins (dolastatin derivatives) using ADC technology, a now well-known strategy for targeted cancer therapy (Doronina et al., 2003; Maderna and Leverett, 2015; Singh, 2022). Brentuximab vedotin (Adcetris®) is an ADC composed by monomethyl auristatin E, a synthetic analogue of dolastatin 10, conjugated with monoclonal antibody CD30, and in 2011 it was approved by the FDA for the treatment of anaplastic large T-cell systemic malignant lymphoma and Hodgkin lymphoma (Senter and Sievers, 2012).

Lyngbyabellins (Fig. 4) were also first isolated from the sea hare *Dolabella auricularia* as dolabellins (Sone et al., 1995) and then attributed to the marine cyanobacterium *Lyngbya majuscula* (Luesch et al., 2000a; Luesch et al., 2000b) and later also from *Moorea* and *Okeania* genera (Fathoni et al., 2020). As mentioned before, due to their ability to disrupt microfilament networks, they were reported to induce apoptosis in several cancer cell lines, with IC_{50} values at low micromolar ranges (Williams et al., 2003; Han et al., 2005; Choi et al., 2012).

Among cyclopeptide cyanotoxins, lagunamides are still under investigation for a full understanding of their mechanism of action. In 2010, lagunamide A (Fig. 12) and B were isolated from *Lyngbya majuscula*, showing potent cytotoxic activity against the P388 murine leukemia cell line, with IC_{50} values of 6.4 and 20.5 nM, respectively (Tripathi et al., 2010). Later, both were proved to show more specific cytotoxicity against a panel of cancer cell lines, with the HCT8 human ileocecal adenocarcinoma cell line as the most sensitive (IC_{50} values of 1.6 and 5.2 nM). Moreover, preliminary biochemical studies suggested that lagunamides might exert their cytotoxicity via induction of mitochondrial mediated apoptosis (Tripathi et al., 2012). More recently, Luo and colleagues isolated lagunamide D from a collection of marine cyanobacteria, mainly composed by *Dichothrix*, *Lyngbya* and *Rivularia* species, which showed anti-proliferative effect against A549 human lung adenocarcinoma cells (IC_{50} values of 7.1 $\pm$ 1.7 nM) (Luo et al., 2019). Lagunamide D, as well as other members of the family, was demonstrated to trigger the apoptosis pathway, by the activation of caspase 3/7, but further investigations on their mechanism of action are still necessary to fully explore the therapeutic potential of lagunamides as anticancer agents.

Among all other cyanotoxins, some of them possess unexplored and unexploited anticancer properties. For example, microcystin analogues are assumed to be selective anticancer drugs for certain types of cancer cells, specifically for those that express organic anion transporting polypeptides (OATPs), without causing significant toxicity to normal cells because of the differences of redox status between normal and cancer cells (Monks et al., 2007; Niedermeyer et al., 2014).

Table 1
List of prominent cyanotoxins with biotechnological applications.

CLASS	TOXIN	MECHANISM	BIOACTIVITY	REFERENCES
alkaloids	anatoxins	nicotinic acetylcholine receptor agonists	larvicide	Berry et al. (2008)
	cylindrospermopsins	inhibition of protein synthesis	larvicide	Berry et al. (2008)
	saxitoxins	sodium channel blockers	local anaesthetic	Epstein-Barash et al. (2009)
indole alkaloids	ambiguines	inhibition of NF-κB pathway	antimicrobial, anticancer	Raveh and Carmeli (2007) Walton and Berry. (2016)
	fischerindoles	inhibition of bacterial RNA polymerase	antimicrobial, anticancer	Kim et al. (2012)
	hapalindoles	inhibition of bacterial RNA polymerase	anticancer, antimicrobial, algaecide, insecticide	Moore et al. (1984) Walton and Berry (2016)
lipoglycans	lyngbyatoxins	activation of protein kinase C	grazing deterrent	Berry et al. (2008)
	welwitindolinones	inhibition of tubulin polymerization	anticancer, antimicrobial, insecticide	Walton and Berry (2016)
	lipopolysaccharides	TLR4 antagonists	immunomodulatory	Jemmett et al. (2008)
	anabaenolysins	disruption of biological membranes	antimicrobial	Shishido et al. (2015)
lipopeptides	antillatoxins	sodium channel activators	neuroplasticity promoter	Mehrotra et al. (2022)
	curacins	microtubule assembly inhibition	anticancer	Gerwick et al. (1994) Yoo and Gerwick (1995) Márquez et al. (1998)
non-ribosomal peptides	anabaenopeptins	inhibition of proteases	antithrombotic	Schreuder et al. (2016)
	aeruginosins	inhibition of proteases	antithrombotic	Del Valle et al. (2014)
	apratoxins	inhibition of cotranslational translocation	anticancer	Luesch et al. (2001a) Gutiérrez et al. (2008) Matthew et al. (2008) Cai et al. (2017)
	cryptophycins	microtubule assembly inhibitor	anticancer	Chen et al. (1998) Lieberman et al. (2001)
	dolastatins	microtubule assembly inhibitor	anticancer	Bai et al. (1992) Poncet (1999)Luesch et al. (2001b)
	lagunamides	mitochondria-mediated apoptosis	anticancer	Tripathi et al. (2010) Luo et al. (2019)
	lyngbyabellins	depolymerisation of actin microfilaments	anticancer, antimicrobial, antifouling	Williams et al. (2003) Han et al. (2005) Choi et al. (2012)
organophosphates	microcystins	inhibition of protein phosphatases	algaecide, larvicide, herbicide	Berry et al. (2008)
	nodularins	inhibition of protein phosphatases	larvicide	Berry et al. (2008)
	guanitoxin	irreversible inhibition of acetylcholinesterase	insecticide	Fiore et al. (2020)
polyketides	aplysiatoxins	activation of protein kinase C, potassium channel blockers	anticancer	Antal et al. (2015) Isakov (2018)
	scytophycins	depolymerisation of actin microfilaments	antimicrobial	Ishibashi et al. (1986) Wang et al. (2017)

Aplysiatoxins (Fig. 5) have been shown to act as PKC activators, with a subsequent tumor-promoting effect that is phorbol esters-like (Nakamura et al., 1989). Although in normal cells the PKC over-activation leads to malignant cell transformation, some human cancers are associated with loss-of-function mutations of specific PKC genes. It was indeed reported that the correction by CRISPR-mediated genome editing of these mutations induced a reduction in tumor growth (Antal et al., 2015; Isakov, 2018). These enlightening studies therefore demonstrated that PKC plays the role of tumor suppressor, thus suggesting that future clinical efforts should focus on stimulating, rather than inhibiting, PKC activity. With this awareness, the role of aplysytoxins in carcinogenesis should be re-analyzed.

3.2. Antimicrobial

The first evidence of cyanobacterial antimicrobial activity dates back to 1917, when the German botanist Richard Harder reported the capability of *Nostoc punctiforme* to excrete toxic agents, endowed with autoinhibiting properties and antibacterial activity (Harder, 1917). Thenceforth, many published papers reported organic extracts and culture supernatants of cyanobacteria exhibiting significant antibacterial, antifungal and antiviral effects (Flint and Moreland, 1946; Burkholder et al., 1960; Starr et al., 1962; Welch, 1962; Patterson et al., 1994; Falch et al., 1995; Dussault et al., 2016; Swain et al., 2017). Nevertheless, only a few antimicrobial compounds from cyanobacteria have been isolated and structurally characterized. Among these, indole alkaloid cyanotoxins from Stigonematales were proved to possess a wide range of biological activities and are now considered potential candidates for novel drug discovery. In particular, hapalindole A (Fig. 13) was first isolated in 1984 from *Hapalosiphon fontinalis*, a chlorine- and isonitrile-containing indole alkaloid which showed interesting antibacterial activity against different *Staphylococcus*, *Streptococcus*, *Salmonella* and *Klebsiella* strains, and antimycotic effects against *Candida albicans* and *Trichophyton mentagrophytes* (Moore et al., 1984; Moore et al., 1987). Hapalindoles, as mentioned before, represent the biosynthetic precursor of other indole alkaloid subclasses, and namely ambiguines, fischerindoles and welwitindolinones (Walton and Berry, 2016; Nandagopal et al., 2021). Each has multiple bioactivities with applications in the industrial and pharmaceutical fields, recently reported in an extensive review on hapalindole-like cyanobacterial alkaloids (Hohlman and Sherman, 2021).

As an example, ambiguine isonitriles (Fig. 13) isolated from *Fischerella* sp. were reported to have antibacterial activity against *Mycobacterium tuberculosis, Bacillus anthracis, Staphylococcus aureus* (MIC 1.0–61.2 μM), and antifungal activity against *Candida albicans*, with MIC values at low micromolar ranges (Smitka et al., 1992; Raveh and Carmeli, 2007). Fischerindole L (Fig. 13), initially isolated from an antifungal extract obtained from *Fischerella muscicola*, was confirmed to inhibit *C. albicans* growth with a MIC value of 1.2 μM and, in addition, it demonstrated a clear antibacterial effect against *Mycobacterium tuberculosis, Mycobacterium smegmatis* and *Staphylococcus aureus* (Park et al., 1992; Kim et al., 2012). N-Methylwelwitindolinone C isothiocyanate (Fig. 13) was responsible for the antifungal effect exerted by *Hapalosiphon welwitschii* lipophilic extract against *Asperigillus oryrae*, *Penicillium notatum*, *Saccharomyces cereuisiae*, and *Trichophyton mentagrophytes* (Stratmann et al., 1994).

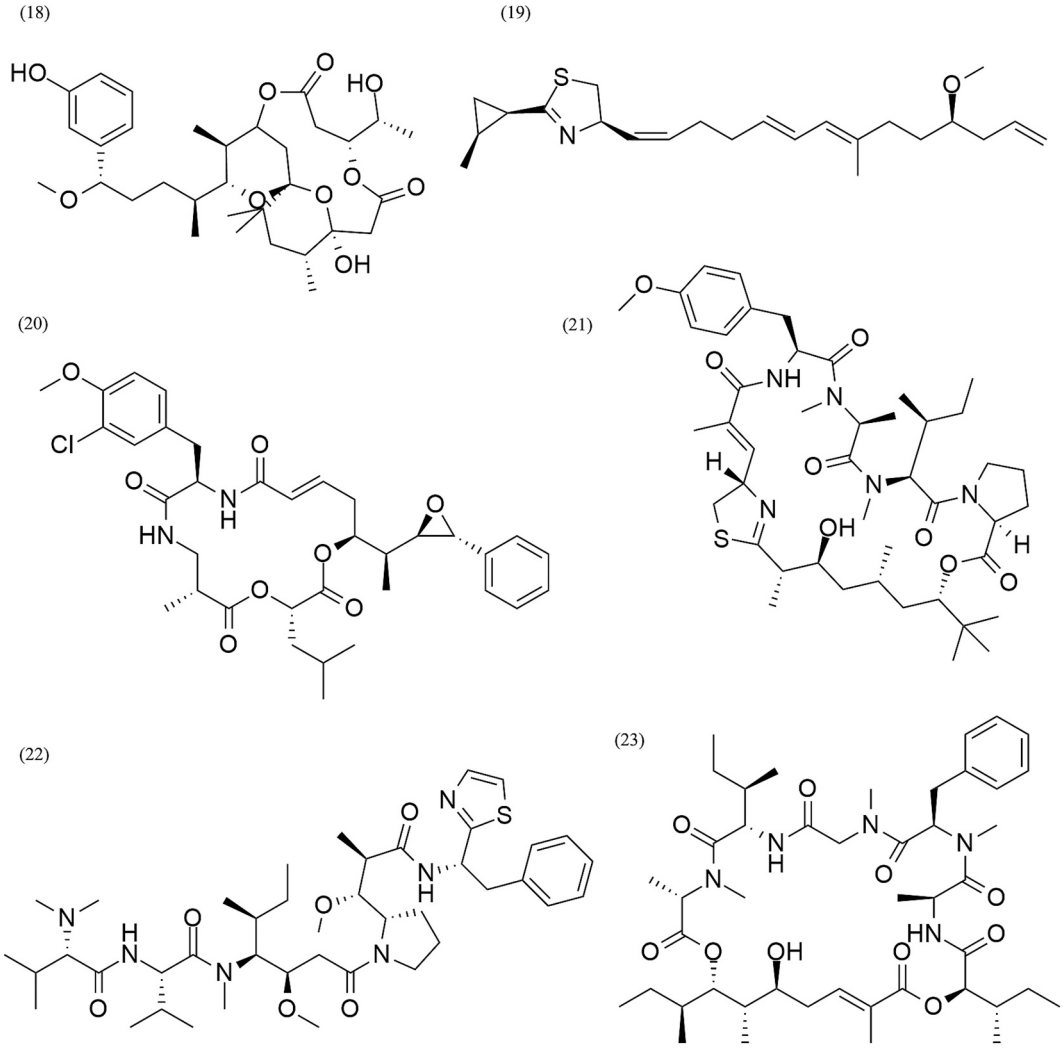

Fig. 12. Chemical structures of anticancer cyanotoxins: 18) debromoaplysiatoxin, 19) curacin A, 20) cryptophycin 1, 21) apratoxin, 22) dolastatin 10, 23) lagunamide A.

Whereas hapalindole-like alkaloids exert their antimicrobial activity by inhibiting RNA polymerase (Doan et al., 2001), other antimicrobial cyanotoxins act by destructuring microbial cells, thus inducing their lysis. This is the case of anabaenolysins (Fig. 9), cytolytic lipopeptides that were supposed to provoke membrane permeabilization in an ergosterol-dependent manner. Their antifungal activity, alone and in combination with cyclodextrins, was demonstrated by disk diffusion assay on *Candida albicans* and *Aspergillus* spp. (Shishido et al., 2015).

Cytotoxic effects of lyngbyabellins (Fig. 4) and scytophycins (Fig. 13), instead, is related to their ability to disrupt the actin microfilament network and, consequently, to block cell division (Han et al., 2005; Smith et al., 1993). This should presumably be the antifungal mechanism of action of both these cyanobacteria-derived macrolides, but no evidence has been reported so far (Ishibashi et al., 1986; Moore et al., 1986; Milligan et al., 2000; Wang et al., 2017).

In some other cases, the mechanism of cytotoxicity has no relation to the beneficial mode of action of cyanotoxins. As an example, antillatoxin (Fig. 9), which is a neurotoxic voltage-gated sodium channel activator, showed antibacterial effects against *Bacillus cereus*, *Staphylococcus aureus* and *Listeria monocytogenes* (MIC = 130–250 μg/ml) (Dussault et al., 2016). However, despite the frantic search for antimicrobials from natural sources that started in the 1940s, many screening studies did not have a follow-up, and indeed no antimicrobials isolated from cyanobacteria have entered clinical trials.

3.3. Biocides

Cyanobacteria produce numerous metabolites with inhibitory and cytotoxic activities against other microorganisms, mammals, fish, crustaceans. Very often, the release of these allelochemicals allows cyanobacteria to avoid planktivorous grazers, or to inhibit the growth of sympatric algal species, potential competitors for nutrients (Berry et al., 2008). Therefore, over the years the possible use of cyanotoxins in the development of biocides has been evaluated. Algaecides, herbicides and insecticides are the products in which cyanotoxins could be widely used, but often their mode of action and potential ecological impact precludes many of them for use as biologically active agents. A clear example is the case of guanitoxin (Fig. 8), a natural occurring organophosphate similar in structure to synthetic nerve agent pesticides and insecticides (Fiore et al., 2020). Since their potent anticholinesterase activity has been correlated to an acute neurological toxicity, in 1993 the Chemical Weapons Convention prohibited the development, production, stockpiling and use of synthetic organophosphate nerve agents (like Sarin, Tabun and Soman), because considered as weapon of mass destruction (CWC, 2020). Therefore, due to its high structural similarity with this class of compounds, the GNT potential application as pesticide was never investigated.

On the contrary, anatoxin-a (Fig. 1) uses have been widely explored as mosquito larvicides. Despite its high cytotoxicity and poisoning

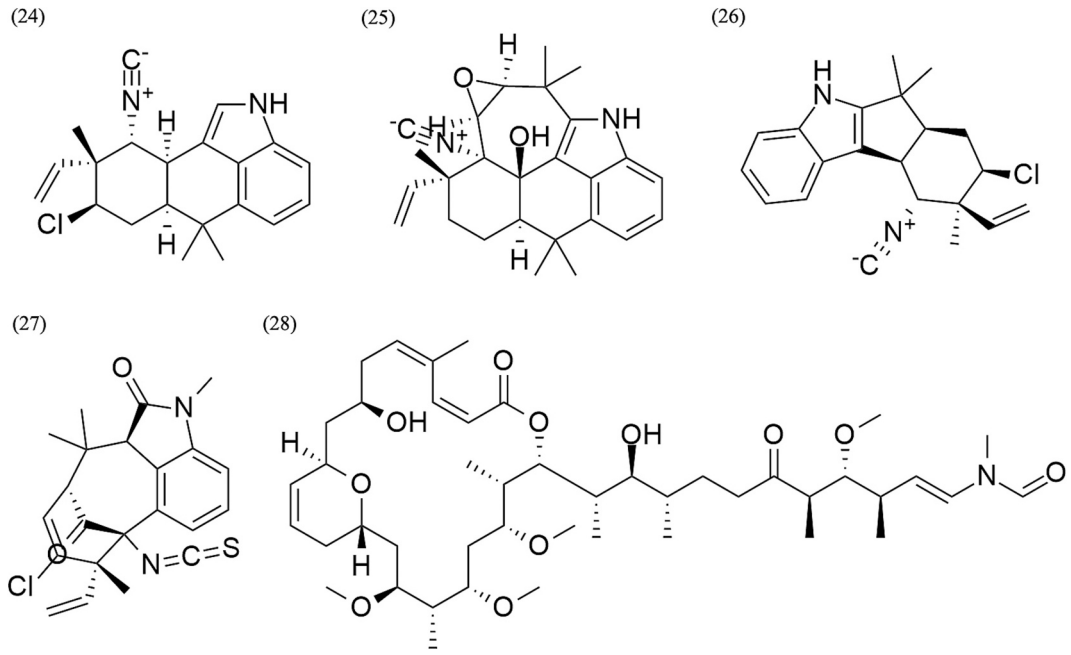

Fig. 13. Chemical structures of antimicrobial cyanotoxins: 24) hapalindole A, 25) ambiguine I isonitrile, 26) fischerindole L, 27) N-methylwelwitindolinone C isothiocyanate, 28) scytophycin.

effects on human and animals (Colas et al., 2021), which would make it an unsuitable candidate for control agent development, it has a short half-life, from 1 to 2 h to 14 days (WHO, 2020a; Sivonen and Jones, 1999), compared to the synthetic insecticide DDT (dichloro-diphenyl-trichloroethane). DDT was first used to control mosquito populations during the Second World War and then entered the market in 1945, soon becoming an effective and cheap insecticide extensively used throughout the world. However, its chemical stability (half-life of about eight years) and lipophilicity resulted in a high bioaccumulation of DDT through the food chain and a long persistence in the environment. In addition, a possible relationship between DDT exposure and increase in cancer risk in humans has been hypothesized, even if not fully confirmed (Beard and Australian Rural Health Research Collaboration, 2006). As a result, DDT was classified as a probable human carcinogen by international authorities and its use was increasingly restricted or banned in most developed countries after 1970. Anyway, the possibility of using anatoxin-a as an insecticide was confirmed when the pure molecule was tested on *Aedes aegypti* mosquito larvae, showing a mortality rate of 50% at concentrations of 50 μg/ml (Kiviranta et al., 1993; Berry, 2014). Unfortunately, considering the potential non-target toxicity, these findings were not sufficient to promote anatoxin-a use in commercial products.

To date, the most promising biocidal strategy is based on the use of cyanotoxins inhibiting the photosynthesis as algaecidal agents. Hapalindole A (Fig. 7) and other indole alkaloids isolated from Nostocales and Stigonematales showed potent antialgal activities, specifically inhibiting photosystem II in algae and other photosynthetic organisms (Moore et al., 1984; Doan et al., 2000; Walton and Berry, 2016). The added value of this type of approach is the exploitation of the innate ability of cyanobacteria to produce target-specific allelochemicals, devoid of non-specific toxicity, especially directed against humans and animals. On the other hand, a drawback of using biocidal agents specifically directed against photoautotrophs is the risk of affecting also non-pest plants. In any case Anyway, although there are numerous candidates with potential biocidal activity, no cyanotoxin has been yet identified which possesses the specific activity required for the development of algaecides, herbicides or insecticides.

3.4. Other applications for clinical purposes

Since there are countless different applications that cyanotoxins can have in medicine, below we report only some other compounds that are considered very promising potential drugs, due to their specific features. Much of the non-ribosomal peptide cyanotoxins are protease inhibitors. In particular, these toxins are able to inhibit the hydrolytic activity of several serine proteases, including elastase, trypsin, thrombin and chymotrypsin, whose deregulation are often involved in various disease conditions like inflammation, atherosclerosis, coagulation abnormalities, pulmonary, neuronal or immunological disorders (Rachel and Sirisha, 2017). In the medical field, anabaenopeptins (APs) (Fig. 4) were reported to be excellent candidates for the development of new drugs for the prevention and treatment of thrombotic diseases. More specifically, APs inhibit the Thrombin Activatable Fibrinolysis Inhibitor (TAFI), a proteolytic enzyme playing a crucial role in haemostasis that represents a possible risk factor for thrombotic and cardiovascular disorders (Halland et al., 2015). Its activated form (TAFIa) acts by cleaving C-terminal lysine residues from fibrin, which are binding sites for plasminogen and tissue plasminogen activator (tPA); their removal induces a reduction of plasmin formation and thus attenuation of fibrinolysis. Consequently, TAFIa inhibition by APs provokes an increase in plasmin generation and fibrin clot degradation, thus resulting in an antithrombotic effect (Schreuder et al., 2016). Another promising cyanotoxin family of peptides endowed with antithrombotic activity are the aeruginosins, which directly inhibit thrombin and other serine proteases such as trypsin and chymotrypsin (Del Valle et al., 2014). Although several aeruginosins exhibiting potent and selective inhibition of thrombin have been identified (Ishida et al., 1999), none of them are currently under evaluation for the treatment of coagulation disorders and thromboembolic disease. However, the discovery of aeruginosins family has paved the way for the study of close natural analogues and the design of peptidomimetics with thrombin inhibiting activity as novel antithrombotic drugs.

As mentioned above, antillatoxins (Fig. 9) work as activators of VGSCs, thus triggering an influx of Na^+ in neurons. Intracellular Na^+ acts, in turn, as a signalling molecule, stimulating ionotropic glutamate receptors (NMDAR), increasing both channel open probability and mean open time. The subsequent increase of intracellular Ca^{2+} provokes the downstream engagement of the Ca^{2+}-dependent CaMKK pathway,

responsible for the induction of neurite outgrowth (Jabba et al., 2010). More recently, Mehrotra and colleagues demonstrated that Ca^{2+} influx also increases brain-derived neurotrophic factor (BDNF) release and the subsequent activation of tropomyosin receptor kinase B (TrkB) signalling, implicated in neuronal and synaptic maturation. These results suggest that VGSC activators like antillatoxin may represent a new pharmacological strategy to promote neuronal plasticity through a NMDAR-BDNF-TrkB-dependent mechanism (Mehrotra et al., 2022). Conversely, saxitoxin (Fig. 1) was demonstrated to induce a potent and prolonged local anaesthesia by VGSCs inhibition and subsequent blocking of axonal conduction propagation. However, due to its innate systemic toxicity, saxitoxin has been listed under the international Chemical Weapons Convention (CAS Number: 35523–89-8) (CWC, 2020), therefore it has never been introduced into clinical practice (Adams et al., 1976; Kohane et al., 2000). Later, the development of saxitoxin-loaded liposomes prolonged the duration of the anaesthetic effect and minimized myotoxicity, neurotoxicity, inflammation, and systemic toxicity (Epstein-Barash et al., 2009).

Last but not least, it is worth mentioning the potential role of cyanobacterial LPSs as anti-inflammatory and immunomodulatory agents. More specifically, an LPS derived from the cyanobacterium *Planktothrix* sp. FP1 (named Cyp) was reported to act as a selective TLR4–MD-2 receptor antagonist. By TLR4 binding, but not activation, Cyp showed an antagonistic effect against *Neisseria meningitidis* lipopolysaccharide, and inhibited cytokine production in an *in vitro* model of septicaemia (Macagno et al., 2006; Jemmett et al., 2008). A partial structure for *Oscillatoria planktothrix* FP1 LPS was also proposed (Carillo et al., 2014), but further studies are necessary to further elucidate the correlation between cyanobacterial LPS structures and their potential clinical applications.

4. Conclusions

Evidence that cyanobacteria represent a valuable reservoir of bioactive natural compounds is clear and widely accepted. Among these precious biomolecules, cyanotoxins are largely produced by cyanobacteria to assist in the normal functioning of basic metabolism or, more frequently, to control secondary functions involved in cell-cell communication, competition for nutrients or adaptation to environmental conditions. Over the years, hundreds of cyanotoxins have been isolated and characterized, and their biological activities studied from an ecological and toxicological point of view. Only in recent decades, thanks to their potential exploitation in the biotechnological field, cyanotoxins have gained more attention, finding applications as biocides, anticancer agents, and antimicrobials. Despite their potent biological activities, a few cyanotoxins have entered clinical trials and far fewer have been approved by the U.S. Food and Drug Administration. This demonstrates how little is still known about the potential of cyanotoxins in the medical and industrial fields. As discussed above, the beneficial role of cyanobacteria deserves more scientific attention and interdisciplinary research. Hopefully, this study can lay the groundwork for a re-evaluation of cyanotoxins, for too long considered only for their toxicity, for innovative biotechnological applications.

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CRediT authorship contribution statement

Annarita Ricciardelli: Conceptualization, Writing – original draft, Writing – review & editing, Visualization. **Antonino Pollio:** Conceptualization, Writing – review & editing, Supervision. **Maria Costantini:** Conceptualization, Writing – review & editing, Supervision. **Valerio Zupo:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Abdallah, M.F., Van Hassel, W., Andjelkovic, M., Wilmotte, A., Rajkovic, A., 2021. Cyanotoxins and food contamination in developing countries: review of their types, toxicity, analysis, occurrence and mitigation strategies. Toxins. 13 (11), 786. https://doi.org/10.3390/toxins13110786.

Abed, R.M., Dobretsov, S., Sudesh, K., 2009. Applications of cyanobacteria in biotechnology. J. Appl. Microbiol. 106 (1), 1–12. https://doi.org/10.1111/j.1365-2672.2008.03918.x.

Adams, H.J., Blair Jr., M.R., Takman, B.H., 1976. The local anesthetic activity of saxitoxin alone and with vasoconstrictor and local anesthetic agents. Arch. Int. Pharmacodyn. Ther. 224 (2), 275–282.

Alcorta, J., Alarcón-Schumacher, T., Salgado, O., Díez, B., 2020. Taxonomic novelty and distinctive genomic features of hot spring cyanobacteria. Front. Genet. 11, 568223 https://doi.org/10.3389/fgene.2020.568223.

Aniszewski, T., 2015. Applied potential and current applications of alkaloids - Chapter 6. In: Aniszewski, T. (Ed.), Alkaloids: Chemistry, Biology, Ecology and Appplications, Second ed. Elsevier B.V, Helsinki, Finland, pp. 345–420. https://doi.org/10.1016/B978-0-444-59433-4.00006-7.

Anselmi, M., Borbély, A., Figueras, E., Michalek, C., Kemker, I., Gentilucci, L., Sewald, N., 2021. Linker hydrophilicity modulates the anticancer activity of RGD-cryptophycin conjugates. Chemistry. 27 (3), 1015–1022. https://doi.org/10.1002/chem.202003471.

Antal, C.E., Hudson, A.M., Kang, E., Zanca, C., Wirth, C., Stephenson, N.L., Trotter, E.W., Gallegos, L.L., Miller, C.J., Furnari, F.B., Hunter, T., Brognard, J., Newton, A.C., 2015. Cancer-associated protein kinase C mutations reveal kinase's role as tumor suppressor. Cell. 160 (3), 489–502. https://doi.org/10.1016/j.cell.2015.01.001.

van Apeldoorn, M.E., van Egmond, H.P., Speijers, G.J., Bakker, G.J., 2007. Toxins of cyanobacteria. Mol. Nutr. Food Res. 51 (1), 7–60. https://doi.org/10.1002/mnfr.200600185.

Aráoz, R., Molgó, J., Tandeau de Marsac, N., 2010. Neurotoxic cyanobacterial toxins. Toxicon. 56 (5), 813–828. https://doi.org/10.1016/j.toxicon.2009.07.036.

Armada-Moreira, A., Gomes, J.I., Pina, C.C., Savchak, O.K., Gonçalves-Ribeiro, J., Rei, N., Pinto, S., Morais, T.P., Martins, R.S., Ribeiro, F.F., Sebastião, A.M., Crunelli, V., Vaz, S.H., 2020. Going the extra (Synaptic) Mile: excitotoxicity as the road toward neurodegenerative diseases. Front. Cell. Neurosci. 14, 90. https://doi.org/10.3389/fncel.2020.00090.

Arnold, A., Edgren, D.C., Palladino, V.S., 1953. Amyotrophic lateral sclerosis; fifty cases observed on Guam. J. Nerv. Ment. Dis. 117 (2), 135–139.

de Arruda, M., Cocchiaro, C.A., Nelson, C.M., Grinnell, C.M., Janssen, B., Haupt, A., Barlozzari, T., 1995. LU103793 (NSC D-669356): a synthetic peptide that interacts with microtubules and inhibits mitosis. Cancer Res. 55 (14), 3085–3092.

Bai, R., Pettit, G.R., Hamel, E., 1990. Dolastatin 10, a powerful cytostatic peptide derived from a marine animal. Inhibition of tubulin polymerization mediated through the vinca alkaloid binding domain. Biochem. Pharmacol. 39 (12), 1941–1949. https://doi.org/10.1016/0006-2952(90)90613-p.

Bai, R., Friedman, S.J., Pettit, G.R., Hamel, E., 1992. Dolastatin 15, a potent antimitotic depsipeptide derived from Dolabella auricularia. Interaction with tubulin and effects of cellular microtubules. Biochem. Pharmacol. 43 (12), 2637–2645. https://doi.org/10.1016/0006-2952(92)90153-a.

Bai, R., Schwartz, R.E., Kepler, J.A., Pettit, G.R., Hamel, E., 1996. Characterization of the interaction of cryptophycin 1 with tubulin: binding in the Vinca domain, competitive inhibition of dolastatin 10 binding, and an unusual aggregation reaction. Cancer Res. 56 (19), 4398–4406.

Beard, J., Australian Rural Health Research Collaboration, 2006. DDT and human health. Sci. Total Environ. 355 (1–3), 78–89. https://doi.org/10.1016/j.scitotenv.2005.02.022.

Berman, F.W., Gerwick, W.H., Murray, T.F., 1999. Antillatoxin and kalkitoxin, ichthyotoxins from the tropical cyanobacterium *Lyngbya majuscula*, induce distinct temporal patterns of NMDA receptor-mediated neurotoxicity. Toxicon. 37 (11), 1645–1648. https://doi.org/10.1016/s0041-0101(99)00108-7.

Berry, G., 2014. Mosquito Larvicides from Cyanobacteria. FIU Electronic Theses and Dissertations N° 1449. https://doi.org/10.25148/etd.FI14071114.

Berry, J.P., Gantar, M., Perez, M.H., Berry, G., Noriega, F.G., 2008. Cyanobacterial toxins as allelochemicals with potential applications as algaecides, herbicides and insecticides. Mar. Drugs. 6 (2), 117–146. https://doi.org/10.3390/md20080007.

Billi, D., Baqué, M., Verseux, C., Rothschild, L., de Vera, J.P., 2017. Desert Cyanobacteria: Potential for Space and Earth Applications. In: Stan-Lotter, H.,

Fendrihan, S. (Eds.), Adaption of Microbial Life to Environmental Extremes. Springer, Cham. https://doi.org/10.1007/978-3-319-48327-6_6.
Borbély, A., Figueras, E., Martins, A., Esposito, S., Auciello, G., Monteagudo, E., Di Marco, A., Summa, V., Cordella, P., Perego, R., Kemker, I., Frese, M., Gallinari, P., Steinkühler, C., Sewald, N., 2019. Synthesis and biological evaluation of RGD-cryptophycin conjugates for targeted drug delivery. Pharmaceutics. 11 (4), 151. https://doi.org/10.3390/pharmaceutics11040151.
Bouaïcha, N., Maatouk, I., 2004. Microcystin-LR and nodularin induce intracellular glutathione alteration, reactive oxygen species production and lipid peroxidation in primary cultured rat hepatocytes. Toxicol. Lett. 148 (1–2), 53–63. https://doi.org/10.1016/j.toxlet.2003.12.005.
Bouaïcha, N., Miles, C.O., Beach, D.G., Labidi, Z., Djabri, A., Benayache, N.Y., Nguyen-Quang, T., 2019. Structural diversity, characterization and toxicology of microcystins. Toxins. 11 (12), 714. https://doi.org/10.3390/toxins11120714.
Bruno, M., Ploux, O., Metcalf, J.S., Mejean, A., Pawlik-Skowronska, B., Furey, A., 2016. Anatoxin-a, homoanatoxin-a, and natural analogues. In: Meriluoto, J., Spoof, L., Codd, G.A. (Eds.), Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. Wiley & Sons, pp. 138–147. https://doi.org/10.1002/9781119068761.ch13.
Büdel, B., Dulić, T., Darienko, T., Rybalka, N., Friedl, T., 2016. Cyanobacteria and algae of biological soil crusts. In: Weber, B., Büdel, B., Belnap, J. (Eds.), Biological Soil Crusts: An Organizing Principle in Drylands. Springer, Cham, pp. 55–80. https://doi.org/10.1007/978-3-319-30214-0_4.
Buratti, F.M., Manganelli, M., Vichi, S., Stefanelli, M., Scardala, S., Testai, E., Funari, E., 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. Arch. Toxicol. 91 (3), 1049–1130. https://doi.org/10.1007/s00204-016-1913-6.
Burkholder, P., Burkholder, L., Almodóvar, L., 1960. Antibiotic activity of some marine Algae of Puerto Rico. De Gruyter. 2 (1–2), 149–156. https://doi.org/10.1515/botm.1960.2.1-2.149.
Cagide, E., Becher, P.G., Louzao, M.C., Espiña, B., Vieytes, M.R., Jüttner, F., Botana, L.M., 2014. Hapalindoles from the cyanobacterium fischerella: potential sodium channel modulators. Chem. Res. Toxicol. 27 (10), 1696–1706. https://doi.org/10.1021/tx500188a.
Cai, W., Chen, Q.Y., Dang, L.H., Luesch, H., 2017. Apratoxin S10, a dual inhibitor of angiogenesis and cancer cell growth to treat highly vascularized tumors. ACS Med. Chem. Lett. 8 (10), 1007–1012. https://doi.org/10.1021/acsmedchemlett.7b00192.
Cai, W., Ratnayake, R., Gerber, M.H., Chen, Q.Y., Yu, Y., Derendorf, H., Trevino, J.G., Luesch, H., 2019. Development of apratoxin S10 (Apra S10) as an anti-pancreatic cancer agent and its preliminary evaluation in an orthotopic patient-derived xenograft (PDX) model. Investig. New Drugs 37 (2), 364–374. https://doi.org/10.1007/s10637-018-0647-0.
Cardellina 2nd, J.H., Marner, F.J., Moore, R.E., 1979. Seaweed dermatitis: structure of lyngbyatoxin A. Science. 204 (4389), 193–195. https://doi.org/10.1126/science.107586.
Carillo, S., Pieretti, G., Bedini, E., Parrilli, M., Lanzetta, R., Corsaro, M.M., 2014. Structural investigation of the antagonist LPS from the cyanobacterium Oscillatoria planktothrix FP1. Carbohydr. Res. 388, 73–80. https://doi.org/10.1016/j.carres.2013.10.008.
Carmichael, W.W., 1992. Cyanobacteria secondary metabolites - the cyanotoxins. J. Appl. Bacteriol. 72 (6), 445–459. https://doi.org/10.1111/j.1365-2672.1992.tb01858.x.
Carmichael, W.W., 2001. Health effects of toxin-producing cyanobacteria: "The CyanoHABs". Hum. Ecol. Risk. Assess. 7 (5), 1393–1407. https://doi.org/10.1080/20018091095087.
Carmichael, W.W., Beasley, V., Bunner, D.L., Eloff, J.N., Falconer, I., Gorham, P., Harada, K., Krishnamurthy, T., Yu, M.J., Moore, R.E., et al., 1988. Naming of cyclic heptapeptide toxins of cyanobacteria (blue-green algae). Toxicon. 26 (11), 971–973. https://doi.org/10.1016/0041-0101(88)90195-x.
Chemical Weapons Convention, 2020. Convention on the Prohibitions of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction. https://www.opcw.org/sites/default/files/documents/CWC/CWC_en.pdf.
Chen, B.D., Nakeff, A., Valeriote, F., 1998. Cellular uptake of a novel cytotoxic agent, cryptophycin-52, by human THP-1 leukemia cells and H-125 lung tumor cells. Int. J. Cancer 77 (6), 869–873. https://doi.org/10.1002/(sici)1097-0215(19980911)77:6<869::aid-ijc12>3.0.co;2-x.
Cheung, M.Y., Liang, S., Lee, J., 2013. Toxin-producing cyanobacteria in freshwater: a review of the problems, impact on drinking water safety, and efforts for protecting public health. J. Microbiol. 51, 1–10. https://doi.org/10.1007/s12275-013-2549-3.
Chiu, A.S., Gehringer, M.M., Braidy, N., Guillemin, G.J., Welch, J.H., Neilan, B.A., 2012. Excitotoxic potential of the cyanotoxin β-methyl-amino-L-alanine (BMAA) in primary human neurons. Toxicon. 60 (6), 1159–1165. https://doi.org/10.1016/j.toxicon.2012.07.169.
Choi, H., Mevers, E., Byrum, T., Valeriote, F.A., Gerwick, W.H., 2012. Lyngbyabellins K-N from Two palmyra atoll collections of the Marine Cyanobacterium Moorea bouillonii. Eur. J. Org. Chem. 2012 (27), 5141–5150. https://doi.org/10.1002/ejoc.201200691.
Chorus, I., Bartam, J., 1999. Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Managment. WHO, New York, NY, USA. https://doi.org/10.1201/9781482295061. FN Spon: London, UK.
Christensen, V.G., Khan, E., 2020. Freshwater neurotoxins and concerns for human, animal, and ecosystem health: A review of anatoxin-a and saxitoxin. Sci. Total Environ. 736, 139515 https://doi.org/10.1016/j.scitotenv.2020.139515.
Colas, S., Marie, B., Lance, E., Quiblier, C., Tricoire-Leignel, H., Mattei, C., 2021. Anatoxin-a: overview on a harmful cyanobacterial neurotoxin from the environmental scale to the molecular target. Environ. Res. 193, 110590 https://doi.org/10.1016/j.envres.2020.110590.
Cox, P.A., Banack, S.A., Murch, S.J., 2003. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc. Natl. Acad. Sci. U. S. A. 100 (23), 13380–13383. https://doi.org/10.1073/pnas.2235808100.
Del Valle, J.R., Therrien, E., Hanessian, S., 2014. Aeruginosins as Thrombin Inhibitors. In: Hanessian, S. (Ed.), Natural Products in Medicinal Chemistry. https://doi.org/10.1002/9783527676545.ch10.
Demay, J., Bernard, C., Reinhardt, A., Marie, B., 2019. Natural products from cyanobacteria: focus on beneficial activities. Mar. Drugs. 17 (6), 320. https://doi.org/10.3390/md17060320.
Devlin, J.P., Edwards, O.E., Gorham, P.R., Hunter, N.R., Pike, R.K., Stavric, B., 1977. Anatoxin-a, a toxic alkaloid from Anabaena flos-aquae NRC-44h. Can. J. Chem. 55, 1367–1371. https://doi.org/10.1139/v77-189.
Dias, F., Antunes, J.T., Ribeiro, T., Azevedo, J., Vasconcelos, V., Leão, P.N., 2017. Cyanobacterial allelochemicals but not cyanobacterial cells markedly reduce microbial community diversity. Front. Microbiol. 8, 1495. https://doi.org/10.3389/fmicb.2017.01495.
Doan, N.T., Rickards, R.W., Rotschild, J.M., Smith, G.D., 2000. Allelopathic actions of the alkaloid 12-epi-hapalindole E isonitrile and calothrixin A from Cyanobacteria of the genera Fischerella and Calothrix. J. Appl. Phycol. 12, 409–416. https://doi.org/10.1023/A:1008170007044.
Doan, N.T., Stewart, P.R., Smith, G.D., 2001. Inhibition of bacterial RNA polymerase by the cyanobacterial metabolites 12-epi-hapalindole E isonitrile and calothrixin A. FEMS Microbiol. Lett. 196 (2), 135–139. https://doi.org/10.1111/j.1574-6968.2001.tb10554.x.
Doronina, S.O., Toki, B.E., Torgov, M.Y., Mendelsohn, B.A., Cerveny, C.G., Chace, D.F., DeBlanc, R.L., Gearing, R.P., Bovee, T.D., Siegall, C.B., Francisco, J.A., Wahl, A.F., Meyer, D.L., Senter, P.D., 2003. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat. Biotechnol. 21 (7), 778–784. https://doi.org/10.1038/nbt832.
Drobac, D., Tokodi, N., Simeunović, J., Baltić, V., Stanić, D., Svirčev, Z., 2013. Human exposure to cyanotoxins and their effects on health. Arh. Hig. Rada. Toksikol. 64 (2), 119–130. https://doi.org/10.2478/10004-1254-64-2013-2320.
Durai, P., Batool, M., Choi, S., 2015. Structure and effects of cyanobacterial lipopolysaccharides. Mar. Drugs. 13 (7), 4217–4230. https://doi.org/10.3390/md13074217.
Dussault, D., Vu, K.D., Vansach, T., Horgen, F.D., Lacroix, M., 2016. Antimicrobial effects of marine algal extracts and cyanobacterial pure compounds against five foodborne pathogens. Food Chem. 199, 114–118. https://doi.org/10.1016/j.foodchem.2015.11.119.
Edelman, M.J., Gandara, D.R., Hausner, P., Israel, V., Thornton, D., DeSanto, J., Doyle, L. A., 2003. Phase 2 study of cryptophycin 52 (LY355703) in patients previously treated with platinum based chemotherapy for advanced non-small cell lung cancer. Lung Cancer 39 (2), 197–199. https://doi.org/10.1016/s0169-5002(02)00511-1.
Epstein-Barash, H., Shichor, I., Kwon, A.H., Hall, S., Lawlor, M.W., Langer, R., Kohane, D. S., 2009. Prolonged duration local anesthesia with minimal toxicity. Proc. Natl. Acad. Sci. U. S. A. 106 (17), 7125–7130. https://doi.org/10.1073/pnas.0900598106.
Evans, D.M., Murphy, P.J., 2011. The cylindrospermopsin alkaloids. Alkaloids. Chem. Biol. 70, 1–77. https://doi.org/10.1016/b978-0-12-391426-2.00001-3.
Evans, M.H., 1969. Mechanism of saxitoxin and tetrodotoxin poisoning. Br. Med. Bull. 25 (3), 263–267. https://doi.org/10.1093/oxfordjournals.bmb.a070715.
Falch, B.S., König, G.M., Wright, A.D., Sticher, O., Angerhofer, C.K., Pezzuto, J.M., Bachmann, H., 1995. Biological activities of cyanobacteria: evaluation of extracts and pure compounds. Planta Med. 61 (4), 321–328. https://doi.org/10.1055/s-2006-958092.
Fathoni, I., Petitbois, J.G., Alarif, W.M., Abdel-Lateff, A., Al-Lihaibi, S.S., Yoshimura, E., Nogata, Y., Vairappan, C.S., Sholikhah, E.N., Okino, T., 2020. Bioactivities of Lyngbyabellins from Cyanobacteria of Moorea and Okeania Genera. Molecules. 25 (17), 3986. https://doi.org/10.3390/molecules25173986.
Ferrão-Filho Ada, S., Kozlowsky-Suzuki, B., 2011. Cyanotoxins: bioaccumulation and effects on aquatic animals. Mar. Drugs. 9 (12), 2729–2772. https://doi.org/10.3390/md9122729.
Fewer, D.P., Jokela, J., Heinilä, L., Aesoy, R., Sivonen, K., Galica, T., Hrouzek, P., Herfindal, L., 2021. Chemical diversity and cellular effects of antifungal cyclic lipopeptides from cyanobacteria. Physiol. Plant. 173 (2), 639–650. https://doi.org/10.1111/ppl.13484.
Fiore, M.F., de Lima, S.T., Carmichael, W.W., McKinnie, S.M.K., Chekan, J.R., Moore, B. S., 2020. Guanitoxin, re-naming a cyanobacterial organophosphate toxin. Harmful Algae 92, 101737. https://doi.org/10.1016/j.hal.2019.101737.
Fischer, W.J., Altheimer, S., Cattori, V., Meier, P.J., Dietrich, D.R., Hagenbuch, B., 2005. Organic anion transporting polypeptides expressed in liver and brain mediate uptake of microcystin. Toxicol. Appl. Pharmacol. 203 (3), 257–263. https://doi.org/10.1016/j.taap.2004.08.012.
Flint, L.H., Moreland, C.F., 1946. Antibiosis in the blue–green algae. Am. J. Bot. 33, 218.
Francis, G., 1878. Poisonous Australian lake. Nature. 18, 11–12. https://doi.org/10.1038/018011d0.
Froscio, S.M., Humpage, A.R., Burcham, P.C., Falconer, I.R., 2003. Cylindrospermopsin-induced protein synthesis inhibition and its dissociation from acute toxicity in mouse hepatocytes. Environ. Toxicol. 18 (4), 243–251. https://doi.org/10.1002/tox.10121.
Fujii, M., Sato, Y., Ito, H., Masago, Y., Omura, T., 2012. Monosaccharide composition of the outer membrane lipopolysaccharide and O-chain from the freshwater cyanobacterium Microcystis aeruginosa NIES-87. J. Appl. Microbiol. 113 (4), 896–903. https://doi.org/10.1111/j.1365-2672.2012.05405.x.

Fujiki, H., Suganuma, M., Hakii, H., Bartolini, G., Moore, R.E., Takayama, S., Sugimura, T., 1984. A two-stage mouse skin carcinogenesis study of lyngbyatoxin A. J. Cancer Res. Clin. Oncol. 108 (1), 174–176. https://doi.org/10.1007/BF00390993.
Funari, E., Testai, E., 2008. Human health risk assessment related to cyanotoxins exposure. Crit. Rev. Toxicol. 38 (2), 97–125. https://doi.org/10.1080/10408440701749454.
Gao, G., Wang, Y., Hua, H., Li, D., Tang, C., 2021. Marine antitumor peptide dolastatin 10: biological activity, structural modification and synthetic chemistry. Mar. Drugs. 19 (7), 363. https://doi.org/10.3390/md19070363.
Gaysina, L.A., Saraf, A., Singh, P., 2019. Cyanobacteria in diverse habitats - Chapter 1. In: Mishra, A.K., Tiwari, D.N., Rai, A.N. (Eds.), Cyanobacteria. Academic Press, pp. 1–28. https://doi.org/10.1016/b978-0-12-814667-5.00001-5.
Gerwick, W.H., Proteau, P.J., Nagle, D.G., Hamel, E., Blokhin, A., Slate, D.L., 1994. Structure of curacin A, a novel antimitotic, antiproliferative and brine shrimp toxic natural product from the marine cyanobacterium *Lyngbya majuscula*. J. Organomet. Chem. 59 (6), 1243–1245. https://doi.org/10.1021/jo00085a006.
Gesner-Apter, S., Carmeli, S., 2009. Protease inhibitors from a water bloom of the cyanobacterium Microcystis aeruginosa. J. Nat. Prod. 72 (8), 1429–1436. https://doi.org/10.1021/np900340t.
Glover, W.B., Mash, D.C., Murch, S.J., 2014 Nov. The natural non-protein amino acid N-β-methylamino-L-alanine (BMAA) is incorporated into protein during synthesis. Amino Acids 46 (11), 2553–2559. https://doi.org/10.1007/s00726-014-1812-1.
Gobler, C.J., 2020. Climate Change and Harmful Algal Blooms: Insights and perspective. Harmful Algae 91, 101731. https://doi.org/10.1016/j.hal.2019.101731.
Grauer, F.H., Arnold Jr., H.L., 1961. Seaweed dermatitis. First report of a dermatitis-producing marine alga. Arch. Dermatol. 84, 720–732. https://doi.org/10.1001/archderm.1961.01580170014003.
Gutiérrez, M., Suyama, T.L., Engene, N., Wingerd, J.S., Matainaho, T., Gerwick, W.H., 2008. Apratoxin D, a potent cytotoxic cyclodepsipeptide from papua new guinea collections of the marine cyanobacteria Lyngbya majuscula and Lyngbya sordida. J. Nat. Prod. 71 (6), 1099–1103. https://doi.org/10.1021/np800121a.
Hagenbuch, B., Gui, C., 2008. Xenobiotic transporters of the human organic anion transporting polypeptides (OATP) family. Xenobiotica. 38 (7–8), 778–801. https://doi.org/10.1080/00498250801986951.
Halland, N., Brönstrup, M., Czech, J., Czechtizky, W., Evers, A., Follmann, M., Kohlmann, M., Schiell, M., Kurz, M., Schreuder, H.A., Kallus, C., 2015. Novel small molecule inhibitors of activated Thrombin Activatable Fibrinolysis Inhibitor (TAFIa) from natural product anabaenopeptin. J. Med. Chem. 58 (11), 4839–4844. https://doi.org/10.1021/jm501840b.
Han, B.N., McPhail, K.L., Gross, H., Goeger, D.E., Mooberry, S.L., Gerwick, W.H., 2005. Isolation and structure of five lyngbyabellin derivatives from a Papua New Guinea collection of the marine cyanobacterium *Lyngbya majuscula*. Tetrahedron. 61, 11723–11729. https://doi.org/10.1016/j.tet.2005.09.036.
Harder, R., 1917. Ernährungsphysiologische Untersuchungen an Cyanophyceen, hauptsächlich dem endophytischen *Nostoc punctiforme*. Z. Bot. 9, 145–242.
Hohlman, R.M., Sherman, D.H., 2021. Recent advances in hapalindole-type cyanobacterial alkaloids: biosynthesis, synthesis, and biological activity. Nat. Prod. Rep. 38 (9), 1567–1588. https://doi.org/10.1039/d1np00007a.
Holland, A., Kinnear, S., 2013. Interpreting the possible ecological role(s) of cyanotoxins: compounds for competitive advantage and/or physiological aide? Mar. Drugs. 11 (7), 2239–2258. https://doi.org/10.3390/md11072239.
Horowitz, A.D., Fujiki, H., Weinstein, I.B., Jeffrey, A., Okin, E., Moore, R.E., Sugimura, T., 1983. Comparative effects of aplysiatoxin, debromoaplysiatoxin, and teleocidin on receptor binding and phospholipid metabolism. Cancer Res. 43 (4), 1529–1535.
Huang, K.C., Chen, Z., Jiang, Y., Akare, S., Kolber-Simonds, D., Condon, K., Agoulnik, S., Tendyke, K., Shen, Y., Wu, K.M., Mathieu, S., Choi, H.W., Zhu, X., Shimizu, H., Kotake, Y., Gerwick, W.H., Uenaka, T., Woodall-Jappe, M., Nomoto, K., 2016. Apratoxin A shows novel pancreas-targeting activity through the binding of sec 61. Mol. Cancer Ther. 15 (6), 1208–1216. https://doi.org/10.1158/1535-7163.MCT-15-0648.
Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16 (8), 471–483. https://doi.org/10.1038/s41579-018-0040-1.
Humbert, J., 2009. Toxins of cyanobacteria. In: Gupta, R.C. (Ed.), Handbook of Toxicology of Chemical Warfare Agents. Elsevier Inc. https://doi.org/10.1016/B978-012374484-5.00027-4
Humpage, A.R., Fontaine, F., Froscio, S., Burcham, P., Falconer, I.R., 2005. Cylindrospermopsin genotoxicity and cytotoxicity: role of cytochrome P-450 and oxidative stress. J. Toxicol. Environ. Health. A. 68 (9), 739–753. https://doi.org/10.1080/15287390590925465.
Isakov, N., 2018. Protein kinase C (PKC) isoforms in cancer, tumor promotion and tumor suppression. Semin. Cancer Biol. 48, 36–52. https://doi.org/10.1016/j.semcancer.2017.04.012.
Ishibashi, M., Moore, R.E., Patterson, G.M.L., Xu, C., Clardy, J., 1986. Scytophycins, cytotoxic and antimycotic agents from the cyanophyte *Scytonema pseudohofmanni*. J. Organomet. Chem. 51 (26), 5300–5306. https://doi.org/10.1021/jo00376a047.
Ishida, K., Okita, Y., Matsuda, H., Okino, T., Murakami, M., 1999. Aeruginosins, protease inhibitors from the cyanobacterium *Microcystis aerugi*nosa. Tetrahedron. 55, 10971–10988. https://doi.org/10.1016/S0040-4020(99)00621-3.
Itou, Y., Suzuki, S., Ishida, K., Murakami, M., 1999. Anabaenopeptins G and H, potent carboxypeptidase A inhibitors from the cyanobacterium *Oscillatoria agardhii* (NIES-595). Bioorg. Med. Chem. Lett. 9 (9), 1243–1246. https://doi.org/10.1016/s0960-894x(99)00191-2.
Jabba, S.V., Prakash, A., Dravid, S.M., Gerwick, W.H., Murray, T.F., 2010. Antillatoxin, a novel lipopeptide, enhances neurite outgrowth in immature cerebrocortical neurons through activation of voltage-gated sodium channels. J. Pharmacol. Exp. Ther. 332 (3), 698–709. https://doi.org/10.1124/jpet.109.161802.
Jemmett, K., Macagno, A., Molteni, M., Heckels, J.E., Rossetti, C., Christodoulides, M., 2008. A cyanobacterial lipopolysaccharide antagonist inhibits cytokine production induced by *Neisseria meningitidis* in a human whole-blood model of septicemia. Infect. Immun. 76 (7), 3156–3163. https://doi.org/10.1128/IAI.00110-08.
Jiang, L., Aigret, B., De Borggraeve, W.M., Spacil, Z., Ilag, L.L., 2012. Selective LC-MS/MS method for the identification of BMAA from its isomers in biological samples. Anal. Bioanal. Chem. 403 (6), 1719–1730. https://doi.org/10.1007/s00216-012-5966-y.
Jokela, J., Oftedal, L., Herfindal, L., Permi, P., Wahlsten, M., Døskeland, S.O., Sivonen, K., 2012. Anabaenolysins, novel cytolytic lipopeptides from benthic Anabaena cyanobacteria. PLoS One 7 (7), e41222. https://doi.org/10.1371/journal.pone.0041222.
Kashiwagi, M., Mynderse, J.S., Moore, R.E., Norton, T.R., 1980. Antineoplastic evaluation of Pacific basin marine algae. J. Pharm. Sci. 69 (6), 735–738. https://doi.org/10.1002/jps.2600690636.
Kerksiek, K., Mejillano, M.R., Schwartz, R.E., Georg, G.I., Himes, R.H., 1995. Interaction of cryptophycin 1 with tubulin and microtubules. FEBS Lett. 377 (1), 59–61. https://doi.org/10.1016/0014-5793(95)01271-0.
Khalifa, S.A.M., Shedid, E.S., Saied, E.M., Jassbi, A.R., Jamebozorgi, F.H., Rateb, M.E., Du, M., Abdel-Daim, M.M., Kai, G.Y., Al-Hammady, M.A.M., Xiao, J., Guo, Z., El-Seedi, H.R., 2021. Cyanobacteria-from the oceans to the potential biotechnological and biomedical applications. Mar. Drugs. 19 (5), 241. https://doi.org/10.3390/md19050241.
Kim, H., Lantvit, D., Hwang, C.H., Kroll, D.J., Swanson, S.M., Franzblau, S.G., Orjala, J., 2012. Indole alkaloids from two cultured cyanobacteria, Westiellopsis sp. and *Fischerella muscicola*. Bioorg. Med. Chem. 20 (17), 5290–5295. https://doi.org/10.1016/j.bmc.2012.06.030.
Kiviranta, J., Abdel-Hameed, A., Sivonen, K., Niemelä, S.I., Carlberg, G., 1993. Toxicity of cyanobacteria to mosquito larvae - screening of active compounds. Environ. Toxicol. Water Qual. 8, 63–71. https://doi.org/10.1002/tox.2530080107.
Kobayashi, M., Natsume, T., Tamaoki, S., Watanabe, J., Asano, H., Mikami, T., Miyasaka, K., Miyazaki, K., Gondo, M., Sakakibara, K., Tsukagoshi, S., 1997. Antitumor activity of TZT-1027, a novel dolastatin 10 derivative. Jpn. J. Cancer Res. 88 (3), 316–327. https://doi.org/10.1111/j.1349-7006.1997.tb00383.x.
Kohane, D.S., Lu, N.T., Gökgöl-Kline, A.C., Shubina, M., Kuang, Y., Hall, S., Strichartz, G. R., Berde, C.B., 2000. The local anesthetic properties and toxicity of saxitonin homologues for rat sciatic nerve block in vivo. Reg. Anesth. Pain Med. 25 (1), 52–59. https://doi.org/10.1016/s1098-7339(00)80011-5.
Kvíderová, J., Elster, J., Komárek, J., 2019. Ecophysiology of cyanobacteria in the polar regions - chapter 14. In: Mishra, A.K., Tiwari, D.N., Rai, A.N. (Eds.), Cyanobacteria. Academic Press, pp. 277–302. https://doi.org/10.1016/B978-0-12-814667-5.00014-3.
Lai, Q., Wu, M., Wang, R., Lai, W., Tao, Y., Lu, Y., Wang, Y., Yu, L., Zhang, R., Peng, Y., Jiang, X., Fu, Y., Wang, X., Zhang, Z., Guo, C., Liao, W., Zhang, Y., Kang, T., Chen, H., Yao, Y., Gou, L., Yang, J., 2020. Cryptophycin-55/52 based antibody-drug conjugates: synthesis, efficacy, and mode of action studies. Eur. J. Med. Chem. 199, 112364 https://doi.org/10.1016/j.ejmech.2020.112364.
Latifi, A., Ruiz, M., Zhang, C.C., 2009. Oxidative stress in cyanobacteria. FEMS Microbiol. Rev. 33 (2), 258–278. https://doi.org/10.1111/j.1574-6976.2008.00134.x.
Leão, P.N., Vasconcelos, M.T.S.D., Vasconcelos, V.M., 2009. Allelopathy in freshwater cyanobacteria. Crit. Rev. Microbiol. 35 (4), 271–282. https://doi.org/10.3109/10408410902823705.
Leflaive, J., Ten-Hage, L., 2007. Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. Freshw. Biol. 52, 199–214. https://doi.org/10.1111/j.1365-2427.2006.01689.x.
Lei, Z., Lei, P., Guo, J., Wang, Z., 2022. Recent advances in nanomaterials-based optical and electrochemical aptasensors for detection of cyanotoxins. Talanta. 248, 123607 https://doi.org/10.1016/j.talanta.2022.123607.
Lenz, K.A., Miller, T.R., Ma, H., 2019. Anabaenopeptins and cyanopeptolins induce systemic toxicity effects in a model organism the nematode *Caenorhabditis elegans*. Chemosphere. 214, 60–69. https://doi.org/10.1016/j.chemosphere.2018.09.076.
Lieberman, M.M., Patterson, G.M., Moore, R.E., 2001. In vitro bioassays for anticancer drug screening: effects of cell concentration and other assay parameters on growth inhibitory activity. Cancer Lett. 173 (1), 21–29. https://doi.org/10.1016/s0304-3835(01)00681-4.
Liu, Y., Law, B.K., Luesch, H., 2009. Apratoxin a reversibly inhibits the secretory pathway by preventing cotranslational translocation. Mol. Pharmacol. 76 (1), 91–104. https://doi.org/10.1124/mol.109.056085.
Lu, K., Dempsey, J., Schultz, R.M., Shih, C., Teicher, B.A., 2001. Cryptophycin-induced hyperphosphorylation of Bcl-2, cell cycle arrest and growth inhibition in human H460 NSCLC cells. Cancer Chemother. Pharmacol. 47 (2), 170–178. https://doi.org/10.1007/s002800000210.
Luesch, H., Yoshida, W.Y., Moore, R.E., Paul, V.J., Mooberry, S.L., 2000a. Isolation, structure determination, and biological activity of Lyngbyabellin A from the marine cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. 63 (5), 611–615. https://doi.org/10.1021/np990543q.
Luesch, H., Yoshida, W.Y., Moore, R.E., Paul, V.J., 2000b. Isolation and structure of the cytotoxin lyngbyabellin B and absolute configuration of lyngbyapeptin A from the marine cyanobacterium *Lyngbya majuscula*. J. Nat. Prod. 63 (10), 1437–1439. https://doi.org/10.1021/np000104n.
Luesch, H., Yoshida, W.Y., Moore, R.E., Paul, V.J., Corbett, T.H., 2001a. Total structure determination of apratoxin A, a potent novel cytotoxin from the marine

cyanobacterium *Lyngbya majuscula*. J. Am. Chem. Soc. 123 (23), 5418–5423. https://doi.org/10.1021/ja010453j.
Luesch, H., Moore, R.E., Paul, V.J., Mooberry, S.L., Corbett, T.H., 2001b. Isolation of dolastatin 10 from the marine cyanobacterium Symploca species VP642 and total stereochemistry and biological evaluation of its analogue symplostatin 1. J. Nat. Prod. 64 (7), 907–910. https://doi.org/10.1021/np010049y.
Luesch, H., Harrigan, G.G., Goetz, G., Horgen, F.D., 2002. The cyanobacterial origin of potent anticancer agents originally isolated from sea hares. Curr. Med. Chem. 9 (20), 1791–1806. https://doi.org/10.2174/0929867023369051.
Luesch, H., Chanda, S.K., Raya, R.M., DeJesus, P.D., Orth, A.P., Walker, J.R., Izpisúa Belmonte, J.C., Schultz, P.G., 2006. A functional genomics approach to the mode of action of apratoxin A. Nat. Chem. Biol. 2 (3), 158–167. https://doi.org/10.1038/nchembio769.
Luo, D., Putra, M.Y., Ye, T., Paul, V.J., Luesch, H., 2019. Isolation, structure elucidation and biological evaluation of lagunamide D: a new cytotoxic macrocyclic depsipeptide from marine cyanobacteria. Mar. Drugs. 17 (2), 83. https://doi.org/10.3390/md17020083.
Ma, D., Zou, B., Cai, G., Hu, X., Liu, J.O., 2006. Total synthesis of the cyclodepsipeptide apratoxin A and its analogues and assessment of their biological activities. Chemistry. 12 (29), 7615–7626. https://doi.org/10.1002/chem.200600599.
Macagno, A., Molteni, M., Rinaldi, A., Bertoni, F., Lanzavecchia, A., Rossetti, C., Sallusto, F., 2006. A cyanobacterial LPS antagonist prevents endotoxin shock and blocks sustained TLR4 stimulation required for cytokine expression. J. Exp. Med. 203 (6), 1481–1492. https://doi.org/10.1084/jem.20060136.
Machado, J., Azevedo, J., Vasconcelos, V., Campos, A., 2018. Mode of action and toxicity of major cyanobacterial toxins and corresponding chemical variants. In: Gopalakrishnakone, P., Stiles, B., Alape-Girón, A., Dubreuil, J., Mandal, M. (Eds.), Microbial Toxins. Toxinology. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-6449-1_30.
Maderna, A., Leverett, C.A., 2015. Recent advances in the development of new auristatins: structural modifications and application in antibody drug conjugates. Mol. Pharm. 12 (6), 1798–1812. https://doi.org/10.1021/mp500762u.
Mahmood, N.A., Carmichael, W.W., 1986. The pharmacology of anatoxin-a(s), a neurotoxin produced by the freshwater cyanobacterium Anabaena flos-aquae NRC 525-17. Toxicon. 24 (5), 425–434. https://doi.org/10.1016/0041-0101(86)90074-7.
Mahmood, N.A., Carmichael, W.W., 1987. Anatoxin-a(s), an anticholinesterase from the cyanobacterium Anabaena flos-aquae NRC-525-17. Toxicon. 25 (11), 1221–1227. https://doi.org/10.1016/0041-0101(87)90140-1.
Márquez, B., Verdier-Pinard, P., Hamel, E., Gerwick, W.H., 1998. Curacin D, an antimitotic agent from the marine cyanobacterium *Lyngbya majuscula*. Phytochemistry. 49 (8), 2387–2389. https://doi.org/10.1016/s0031-9422(98)00365-3.
de Marsac, T.N., Houmard, J., 1993. Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol. Lett. 104, 119–189. https://doi.org/10.1111/j.1574-6968.1993.tb05866.x.
Mathe, C., Hamvas, M., Garda, T., Beyer, D., Vasas, G., 2017. Cellular effects of cylindrospermopsin (Cyanobacterial Alkaloid Toxin) and its potential medical consequences. Curr. Med. Chem. 24 (1), 91–109. https://doi.org/10.2174/0929867323666161028153814.
Matsunaga, S., Moore, R.E., Niemczura, W.P., Carmichael, W.W., 1989. Anatoxin-a(s), a potent anticholinesterase from Anabaena flos-aquae. J. Am. Chem. Soc. 111, 8021–8023. https://doi.org/10.1021/ja00202a057.
Matsuura, M., 2013. Structural modifications of bacterial lipopolysaccharide that facilitate gram-negative bacteria evasion of host innate immunity. Front. Immunol. 4, 109. https://doi.org/10.3389/fimmu.2013.00109.
Matthew, S., Schupp, P.J., Luesch, H., 2008. Apratoxin E, a cytotoxic peptolide from a guamanian collection of the marine cyanobacterium *Lyngbya bouillonii*. J. Nat. Prod. 71 (6), 1113–1116. https://doi.org/10.1021/np700717s.
Mazgaeen, L., Gurung, P., 2020. Recent advances in lipopolysaccharide recognition systems. Int. J. Mol. Sci. 21 (2), 379. https://doi.org/10.3390/ijms21020379.
McCoy, R.M., Widhalm, J.R., McNickle, G.G., 2022. Allelopathy as an evolutionary game. Plant Direct. 6 (2), e382 https://doi.org/10.1002/pld3.382.
Mehrotra, S., Pierce, M.L., Cao, Z., Jabba, S.V., Gerwick, W.H., Murray, T.F., 2022. Antillatoxin-stimulated neurite outgrowth involves the Brain-derived Neurotrophic Factor (BDNF) - Tropomyosin Related Kinase B (TrkB) signaling pathway. J. Nat. Prod. 85 (3), 562–571. https://doi.org/10.1021/acs.jnatprod.1c01001.
Metcalf, J.S., Codd, G.A., 2014. A Review of Current Knowledge. Cyanobacterial toxins (cyanotoxins) in water. FR/R0009. Foundation for Water Research, Marlow, Bucks, UK.
Metcalf, J.S., Codd, G.A., 2020. Co-occurrence of cyanobacteria and cyanotoxins with other environmental health hazards: impacts and implications. Toxins. 12 (10), 629. https://doi.org/10.3390/toxins12100629.
Miglione, A., Napoletano, M., Cinti, S., 2021. Electrochemical biosensors for tracing cyanotoxins in food and environmental matrices. Biosensors. 11 (9), 315. https://doi.org/10.3390/bios11090315.
Milligan, K.E., Marquez, B.L., Williamson, R.T., Gerwick, W.H., 2000. Lyngbyabellin B, a toxic and antifungal secondary metabolite from the marine cyanobacterium Lyngbya majuscula. J. Nat. Prod. 63 (10), 1440–1443. https://doi.org/10.1021/np000133y.
Molisch, H., 1937. Der Einfluss Einer Pflanze Auf Die Andere - Allelopathie. Fischer, Jena.
Monks, N.R., Liu, S., Xu, Y., Yu, H., Bendelow, A.S., Moscow, J.A., 2007. Potent cytotoxicity of the phosphatase inhibitor microcystin LR and microcystin analogues in OATP1B1- and OATP1B3-expressing HeLa cells. Mol. Cancer Ther. 6 (2), 587–598. https://doi.org/10.1158/1535-7163.MCT-06-0500.
Monteiro, P.R., do Amaral, S.C., Siqueira, A.S., Xavier, L.P., Santos, A.V., 2021. Anabaenopeptins: what we know so far. Toxins. 13 (8), 522. https://doi.org/10.3390/toxins13080522.
Mooberry, S.L., Busquets, L., Tien, G., 1997. Induction of apoptosis by cryptophycin 1, a new antimicrotubule agent. Int. J. Cancer 73 (3), 440–448. https://doi.org/10.1002/(sici)1097-0215(19971104)73:3<440::aid-ijc20>3.0.co;2-f.
Moore, R.E., Cheuk, C., Patterson, G.M.L., 1984. Hapalindoles: new alkaloids from the blue-green alga Hapalosiphon fontinalis. J. Am. Chem. Soc. 106 (21), 6456–6457. https://doi.org/10.1021/ja00333a079.
Moore, R.E., Patterson, G.M.L., Mynderse, J.S., Barchi, J.J., Norton, T.R., Furusawa, E., Furusawa, S., 1986. Toxins from cyanophytes belonging to the scytonemataceae. Pure Appl. Chem. 58, 263–271. https://doi.org/10.1351/PAC198658020263.
Moore, R.E., Cheuk, C., Yang, X.Q.G., Patterson, G.M.L., Bonjouklian, R., Smitka, T.A., Mynderse, J.S., Foster, R.S., Jones, N.D., 1987. Hapalindoles, antibacterial and antimycotic alkaloids from the cyanophyte *Hapalosiphon fontinalis*. J. Organomet. Chem. 52 (6), 1036–1043. https://doi.org/10.1021/jo00382a012.
Mur, L.R., Skulberg, O.M., Utkilen, H., 1999. Cyanobacteria in the environment - Chapter 2. In: Chorus, I., Welker, M. (Eds.), Toxic Cyanobacteria in Water: a Guide to their Public Health Consequences, Monitoring and Management. CRC Press, pp. 14–40. https://doi.org/10.1201/9781482295061.
Mynderse, J.S., Moore, R.E., Kashiwagi, M., Norton, T.R., 1977. Antileukemia activity in the osillatoriaceae: isolation of Debromoaplysiatoxin from Lyngbya. Science. 196 (4289), 538–540. https://doi.org/10.1126/science.403608.
Nakamura, H., Kishi, Y., Pajares, M.A., Rando, R.R., 1989. Structural basis of protein kinase C activation by tumor promoters. Proc. Natl. Acad. Sci. U. S. A. 86 (24), 9672–9676. https://doi.org/10.1073/pnas.86.24.9672.
Nandagopal, P., Steven, A.N., Chan, L.W., Rahmat, Z., Jamaluddin, H., Mohd Noh, N.I., 2021. Bioactive metabolites produced by cyanobacteria for growth adaptation and their pharmacological properties. Biology. 10 (10), 1061. https://doi.org/10.3390/biology10101061.
Nazari-Sharabian, M., Ahmad, S., Karakouzian, M., 2018. Climate change and eutrophication: a short review. Eng. Technol. Appl. Sci. Res. 8 (6), 3668–3672. https://doi.org/10.48084/etasr.
Neilan, B.A., Dittmann, E., Rouhiainen, L., Bass, R.A., Schaub, V., Sivonen, K., Börner, T., 1999. Nonribosomal peptide synthesis and toxigenicity of cyanobacteria. J. Bacteriol. 181 (13), 4089–4097. https://doi.org/10.1128/JB.181.13.4089-4097.1999.
Niedermeyer, T.H., Daily, A., Swiatecka-Hagenbruch, M., Moscow, J.A., 2014. Selectivity and potency of microcystin congeners against OATP1B1 and OATP1B3 expressing cancer cells. PLoS One 9 (3), e91476. https://doi.org/10.1371/journal.pone.0091476.
Oftedal, L., Myhren, L., Jokela, J., Gausdal, G., Sivonen, K., Døskeland, S.O., Herfindal, L., 2012. The lipopeptide toxins anabaenolysin A and B target biological membranes in a cholesterol-dependent manner. Biochim. Biophys. Acta 1818 (12), 3000–3009. https://doi.org/10.1016/j.bbamem.2012.07.015.
van Onselen, R., Cook, N.A., Phelan, R.R., Downing, T.G., 2015. Bacteria do not incorporate β-N-methylamino-L-alanine into their proteins. Toxicon. 102, 55–61. https://doi.org/10.1016/j.toxicon.2015.05.014.
Orr, P.T., Jones, G.J., 1998. Relationship between microcystin production and cell division rates in nitrogen-limited Microcystis aeruginosa cultures. Limnol. Oceanogr. 43 (7), 1604–1614. https://doi.org/10.4319/lo.1998.43.7.1604.
Osborne, N.J., Webb, P.M., Shaw, G.R., 2001. The toxins of *Lyngbya majuscula* and their human and ecological health effects. Environ. Int. 27 (5), 381–392. https://doi.org/10.1016/s0160-4120(01)00098-8.
Paatero, A.O., Kellosalo, J., Dunyak, B.M., Almaliti, J., Gestwicki, J.E., Gerwick, W.H., Taunton, J., Paavilainen, V.O., 2016. Apratoxin kills cells by direct blockade of the sec61 protein translocation channel. Cell. Chem. Biol. 23 (5), 561–566. https://doi.org/10.1016/j.chembiol.2016.04.008.
Paerl, H.W., 2017. Controlling cyanobacterial harmful blooms in freshwater ecosystems. Microb. Biotechnol. 10 (5), 1106–1110. https://doi.org/10.1111/1751-7915.12725.
Park, A., Moore, R.E., Patterson, G.M.L., 1992. Fischerindole L, a new isonitrile from the terrestrial blue-green alga *Fischerella muscicola*. Tetrahedron Lett. 33 (23), 3257–3260. https://doi.org/10.1016/S0040-4039(00)92061-6.
Parthasarathy, A., Borrego, E.J., Savka, M.A., Dobson, R.C.J., Hudson, A.O., 2021. Amino acid-derived defense metabolites from plants: aA potential source to facilitate novel antimicrobial development. J. Biol. Chem. 296, 100438 https://doi.org/10.1016/j.jbc.2021.100438.
Patočka, J., Gupta, R.C., Kuča, K., 2011. Anatoxin-a(s): natural organophosphorus anticholinesterase agent. Mil. Med. Sci. Lett. 80, 129–139. https://doi.org/10.31482/mmsl.2011.019.
Patterson, G.M.L., Baldwin, C.L., Bolis, C.M., Caplan, F.R., Karuso, H., Larsen, L.K., Levine, I.A., Moore, R.E., Nelson, C.S., Tschappat, K.D., Tuang, G.D., Furusawa, E., Furusawa, S., Norton, T.R., Raybourne, R.B., 1991. Antineoplastic activity of cultured blue-green algae (cyanophyta). J. Phycol. 27, 530–536. https://doi.org/10.1111/j.0022-3646.1991.00530.x.
Patterson, G.M.L., Larsen, L.K., Moore, R.E., 1994. Bioactive natural products from blue-green algae. J. Appl. Phycol. 6, 151–157. https://doi.org/10.1007/BF02186069.
Pearson, L.A., Dittmann, E., Mazmouz, R., Ongley, S.E., D'Agostino, P.M., Neilan, B.A., 2016. The genetics, biosynthesis and regulation of toxic specialized metabolites of cyanobacteria. Harmful Algae 54, 98–111. https://doi.org/10.1016/j.hal.2015.11.002.
Pelletier, S.W., 1983. Alkaloids: Chemical and Biological Perspectives. John Wiley & Sons, New York, pp. 1–31.
Pettit, G.R., Kamano, Y., Herald, C.L., Fujii, Y., Kizu, H., Boyd, M.R., Boettner, F.E., Doubek, D.L., Schmidt, J.M., Chapuis, J., Michel, C., 1993. Isolation of dolastatins

10-15 from the marine mollusc dolabella auricularia. Tetrahedron. 49 (41), 9151–9170. https://doi.org/10.1016/0040-4020(93)80003-C.
Plaas, H.E., Paerl, H.W., 2021. Toxic cyanobacteria: a growing threat to water and air quality. Environ. Sci. Technol. 55 (1), 44–64. https://doi.org/10.1021/acs.est.0c06653.
Poncet, J., 1999. The dolastatins, a family of promising antineoplastic agents. Curr. Pharm. Des. 5 (3), 139–162.
Rachel, K.V., Sirisha, G.V.D., 2017. Serine proteases and their inhibitors in human health and disease. In: Chakraborti, S., Chakraborti, T., Dhalla, N. (Eds.), Proteases in Human Diseases. Springer, Singapore. https://doi.org/10.1007/978-981-10-3162-5_10.
Rai, A.N., Bergman, B., Rasmussen, U., 2002. Cyanobacteria in Symbiosis. Kluwer Academic Publishers, Dordrecht, The Netherlands. https://doi.org/10.1007/0-306-48005-0.
Rao, S.D., Banack, S.A., Cox, P.A., Weiss, J.H., 2006. BMAA selectively injures motor neurons via AMPA/kainate receptor activation. Exp. Neurol. 201 (1), 244–252. https://doi.org/10.1016/j.expneurol.2006.04.017.
Raveh, A., Carmeli, S., 2007. Antimicrobial ambiguines from the cyanobacterium Fischerella sp. collected in Israel. J. Nat. Prod. 70 (2), 196–201. https://doi.org/10.1021/np060495r.
Reinhard, C.T., Planavsky, N.J., Gill, B.C., Ozaki, K., Robbins, L.J., Lyons, T.W., Fischer, W.W., Wang, C., Cole, D.B., Konhauser, K.O., 2017. Evolution of the global phosphorus cycle. Nature. 541 (7637), 386–389. https://doi.org/10.1038/nature20772.
Rice, E.L., 1984. Allelopathy, 2nd ed. Academic Press, Orlando.
Rinehart, K.L., Harada, K., Namikoshi, M., Chen, C., Harvis, C.A., Munro, M.H.G., Blunt, J.W., Mulligan, P.E., Beasley, V.R., Dahlem, A.M., Carmicheal, W.W., 1988. Nodularin, microcystin, and the configuration of Adda. J. Am. Chem. Soc. 110, 8557–8558. https://doi.org/10.1021/ja00233a049.
Sano, T., Usui, T., Ueda, K., Osada, H., Kaya, K., 2001. Isolation of new protein phosphatase inhibitors from two cyanobacteria species, Planktothrix spp. J. Nat. Prod. 64 (8), 1052–1055. https://doi.org/10.1021/np0005356.
Sanseverino, I., Conduto António, D., Loos, R., Lettieri, T., 2017. Cyanotoxins: methods and approaches for their analysis and detection. EUR 28624 EN. Publications Office of the European Union, Luxembourg (Luxembourg). JRC106478.
Schirrmeister, B.E., Sanchez-Baracaldo, P., Wacey, D., 2016. Cyanobacterial evolution during the Precambrian. Int. J. Astrobiol. 15 (3), 187–204. https://doi.org/10.1017/S1473550415000579.
Schreuder, H., Liesum, A., Lönze, P., Stump, H., Hoffmann, H., Schiell, M., Kurz, M., Toti, L., Bauer, A., Kallus, C., Klemke-Jahn, C., Czech, J., Kramer, D., Enke, H., Niedermeyer, T.H.J., Morrison, V., Kumar, V., Brönstrup, M., 2016. Isolation, Co-Crystallization and structure-based characterization of anabaenopeptins as highly potent inhibitors of activated Thrombin Activatable Fibrinolysis Inhibitor (TAFIa). Sci. Rep. 6, 32958. https://doi.org/10.1038/srep32958.
Senter, P.D., Sievers, E.L., 2012. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. Nat. Biotechnol. 30 (7), 631–637. https://doi.org/10.1038/nbt.2289.
Serrà, A., Philippe, L., Gómez, E., 2021. Removal of cyanobacteria and cyanotoxins in waters. Toxins. 13 (9), 636. https://doi.org/10.3390/toxins13090636.
Shishido, T.K., Jokela, J., Kolehmainen, C.T., Fewer, D.P., Wahlsten, M., Wang, H., Rouhiainen, L., Rizzi, E., De Bellis, G., Permi, P., Sivonen, K., 2015. Antifungal activity improved by coproduction of cyclodextrins and anabaenolysins in Cyanobacteria. Proc. Natl. Acad. Sci. U. S. A. 112 (44), 13669–13674. https://doi.org/10.1073/pnas.1510432112.
Singh, R., Sharma, M., Joshi, P., Rawat, D.S., 2008. Clinical status of anti-cancer agents derived from marine sources. Anti Cancer Agents Med. Chem. 8 (6), 603–617.
Singh, S.B., 2022. Discovery and development of dolastatin 10-derived antibody drug conjugate anticancer drugs. J. Nat. Prod. 85 (3), 666–687. https://doi.org/10.1021/acs.jnatprod.1c01135.
Sinha, R.P., Häder, D.P., 2008. UV-protectants in cyanobacteria. Plant Sci. 174, 278–289. https://doi.org/10.1016/j.plantsci.2007.12.004.
Sivonen, K., Jones, G.J., 1999. Cyanobacterial Toxins. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water - A Guide to Their Public Health Consequences, Monitoring, and Management. WHO, New York, NY, USA, pp. 41–111. https://doi.org/10.1201/9781482295061-3. FN Spon: London, UK.
Smith, C.D., Carmeli, S., Moore, R.E., Patterson, G.M., 1993. Scytophycins, novel microfilament-depolymerizing agents which circumvent P-glycoprotein-mediated multidrug resistance. Cancer Res. 53 (6), 1343–1347.
Smith, C.D., Zhang, X., Mooberry, S.L., Patterson, G.M., Moore, R.E., 1994. Cryptophycin: a new antimicrotubule agent active against drug-resistant cells. Cancer Res. 54 (14), 3779–3784.
Smitka, T.A., Bonjouklian, R., Doolin, L., Jones, N.D., Deeter, J.B., Yoshida, W.Y., Prinsep, M.R., Moore, R.E., Patterson, G.M.L., 1992. Ambiguine isonitriles, fungicidal hapalindole-type alkaloids from three genera of blue-green algae belonging to the Stigonemataceae. J. Organomet. Chem. 57, 857–861. https://doi.org/10.1021/jo00029a014.
Snyder, D.S., Brahamsha, B., Azadi, P., Palenik, B., 2009. Structure of compositionally simple lipopolysaccharide from marine synechococcus. J. Bacteriol. 191 (17), 5499–5509. https://doi.org/10.1128/JB.00121-09.
Solomon, A.E., Stoughton, R.B., 1978. Dermatitis from purified sea algae toxin (debromoaplysiatoxin). Arch. Dermatol. 114 (9), 1333–1335.
Sone, H., Kondo, T., Kiryu, M., Ishiwata, H., Ojika, M., Yamada, K., 1995. Dolabellin, a cytotoxic bisthiazole metabolite from the sea hare dolabella auricularia: structural determination and synthesis. J. Organomet. Chem. 60, 4774–4781. https://doi.org/10.1021/JO00120A021.
Spencer, P.S., Nunn, P.B., Hugon, J., Ludolph, A.C., Ross, S.M., Roy, D.N., Robertson, R.C., 1987. Guam amyotrophic lateral sclerosis-parkinsonism-dementia linked to a plant excitant neurotoxin. Science. 237 (4814), 517–522. https://doi.org/10.1126/science.3603037.
Spoof, L., Błaszczyk, A., Meriluoto, J., Cegłowska, M., Mazur-Marzec, H., 2015. Structures and activity of new anabaenopeptins produced by baltic sea cyanobacteria. Mar. Drugs. 14 (1), 8. https://doi.org/10.3390/md14010008.
Starr, T.J., Dieg, E.F., Church, K.K., Allen, M.B., 1962. Antibacterial and antiviral activities of algal extracts studied by acridine orange staining. Tex. Rep. Biol. Med. 20, 271–278.
Stewart, I., Schluter, P.J., Shaw, G.R., 2006. Cyanobacterial lipopolysaccharides and human health - a review. Environ. Health 5, 7. https://doi.org/10.1186/1476-069X-5-7.
Stratmann, K., Moore, R.E., Bonjouklian, R., Deeter, J.B., Patterson, G.M.L., Shaffer, S., Smith, C.D., Smitka, T.A., 1994. Welwitindolinones, unusual alkaloids from the blue-green algae *Hapalosiphon welwitschii* and *Westiella intricata*. Relationship to fischerindoles and hapalinodoles. J. Am. Chem. Soc. 116, 9935–9942. https://doi.org/10.1021/ja00101a015.
Swain, S.S., Paidesetty, S.K., Padhy, R.N., 2017. Antibacterial, antifungal and antimycobacterial compounds from cyanobacteria. Biomed. Pharmacother. 90, 760–776. https://doi.org/10.1016/j.biopha.2017.04.030.
Thottumkara, A.P., Parsons, W.H., Du Bois, J., 2014. Saxitoxin. Angew. Chem. Int. Ed. Eng. 53 (23), 5760–5784. https://doi.org/10.1002/anie.201308235.
Tidgewell, K., Clark, B.R., Gerwick, W.H., 2010. The natural products chemistry of cyanobacteria. In: Mander, L., Liu, H. (Eds.), Comprehensive Natural Products II - Chemistry and Biology. Elsevier Ltd, Amsterdam, The Netherlands, pp. 141–188. https://doi.org/10.1016/B978-008045382-8.00041-1.
Tripathi, A., Puddick, J., Prinsep, M.R., Rottmann, M., Tan, L.T., 2010. Lagunamides A and B: cytotoxic and antimalarial cyclodepsipeptides from the marine cyanobacterium Lyngbya majuscula. J. Nat. Prod. 73 (11), 1810–1814. https://doi.org/10.1021/np100442x.
Tripathi, A., Fang, W., Leong, D.T., Tan, L.T., 2012. Biochemical studies of the lagunamides, potent cytotoxic cyclic depsipeptides from the marine cyanobacterium Lyngbya majuscula. Mar. Drugs. 10 (5), 1126–1137. https://doi.org/10.3390/md10051126.
Vega, A., Bell, E.A., 1967. α-Amino-β-methylaminopropionic acid, a new amino acid from seeds of Cycas circinalis. Phytochemistry. 6, 759–762. https://doi.org/10.1016/S0031-9422(00)86018-5.
Verdier-Pinard, P., Lai, J.Y., Yoo, H.D., Yu, J., Marquez, B., Nagle, D.G., Nambu, M., White, J.D., Falck, J.R., Gerwick, W.H., Day, B.W., Hamel, E., 1998. Structure-activity analysis of the interaction of curacin A, the potent colchicine site antimitotic agent, with tubulin and effects of analogs on the growth of MCF-7 breast cancer cells. Mol. Pharmacol. 53 (1), 62–76. https://doi.org/10.1124/mol.53.1.62.
Verma, V.A., Pillow, T.H., DePalatis, L., Li, G., Phillips, G.L., Polson, A.G., Raab, H.E., Spencer, S., Zheng, B., 2015. The cryptophycins as potent payloads for antibody drug conjugates. Bioorg. Med. Chem. Lett. 25 (4), 864–868. https://doi.org/10.1016/j.bmcl.2014.12.070.
Vidal, L., Ballot, A., Azevedo, S.M.F.O., Padisák, J., Welker, M., 2021. Introduction to cyanobacteria. In: Chorus, I., Welker, M. (Eds.), Toxic Cyanobacteria in Water. CRC Press, Boca Raton, FL, USA, pp. 163–211. https://doi.org/10.1201/9781003081449-3.
Walton, K., Berry, J.P., 2016 Apr 6. Indole alkaloids of the stigonematales (Cyanophyta): chemical diversity, biosynthesis and biological activity. Mar Drugs. 14 (4), 73. https://doi.org/10.3390/md14040073.
Wang, M., Zhang, J., He, S., Yan, X., 2017. A review study on macrolides isolated from cyanobacteria. Mar. Drugs. 15 (5), 126. https://doi.org/10.3390/md15050126.
Wang, S., Jiao, Y., Rao, Z., 2021. Selective removal of common cyanotoxins: a review. Environ. Sci. Pollut. Res. Int. 28 (23), 28865–28875. https://doi.org/10.1007/s11356-021-13798-6.
Weiss, J.H., Christine, C.W., Choi, D.W., 1989. Bicarbonate dependence of glutamate receptor activation by beta-N-methylamino-L-alanine: channel recording and study with related compounds. Neuron. 3 (3), 321–326. https://doi.org/10.1016/0896-6273(89)90256-0.
Welch, A.M., 1962. Preliminary survey of fungistatic properties of marine algae. J. Bacteriol. 83 (1), 97–99. https://doi.org/10.1128/jb.83.1.97-99.1962.
Williams, P.G., Luesch, H., Yoshida, W.Y., Moore, R.E., Paul, V.J., 2003. Continuing studies on the cyanobacterium Lyngbya sp.: isolation and structure determination of 15-norlyngbyapeptin A and lyngbyabellin D. J. Nat. Prod. 66 (5), 595–598. https://doi.org/10.1021/np030011g.
Wipf, P., Reeves, J.T., Balachandran, R., Day, B.W., 2002. Synthesis and biological evaluation of structurally highly modified analogues of the antimitotic natural product curacin A. J. Med. Chem. 45 (9), 1901–1917. https://doi.org/10.1021/jm0105171.
Wipf, P., Reeves, J.T., Day, B.W., 2004. Chemistry and biology of curacin A. Curr. Pharm. Des. 10 (12), 1417–1437. https://doi.org/10.2174/1381612043384853.
Wonnacott, S., Swanson, K.L., Albuquerque, E.X., Huby, N.J., Thompson, P., Gallagher, T., 1992. Homoanatoxin: a potent analogue of anatoxin-A. Biochem. Pharmacol. 43 (3), 419–423. https://doi.org/10.1016/0006-2952(92)90558-z.
World Health Organization, 2020a. Cyanobacterial Toxins: Anatoxin-A and Analogues. WHO. https://apps.who.int/iris/handle/10665/338060 (License: CC BY-NC-SA 3.0 IGO).
World Health Organization, 2020b. Cyanobacterial Toxins: Saxitoxins. WHO. https://apps.who.int/iris/handle/10665/338069 (License: CC BY-NC-SA 3.0 IGO).
World Health Organization, 2020c. Cyanobacterial toxins: cylindrospermopsins. WHO. https://apps.who.int/iris/handle/10665/338063 (License: CC BY-NC-SA 3.0 IGO).

World Health Organization, 2020d. Cyanobacterial Toxins: Microcystins. WHO. https://apps.who.int/iris/handle/10665/338066 (License: CC BY-NC-SA 3.0 IGO).

Yoo, H.D., Gerwick, W.H., 1995. Curacins B and C, new antimitotic natural products from the marine cyanobacterium *Lyngbya majuscule*. J. Nat. Prod. 58 (12), 1961–1965. https://doi.org/10.1021/np50126a029.

Yoshizawa, S., Matsushima, R., Watanabe, M.F., Harada, K., Ichihara, A., Carmichael, W. W., Fujiki, H., 1990. Inhibition of protein phosphatases by microcystins and nodularin associated with hepatotoxicity. J. Cancer Res. Clin. Oncol. 116 (6), 609–614. https://doi.org/10.1007/BF01637082.

Yunes, J.S., 2019. Cyanobacterial Toxins. In: Mishra, A.K., Tiwari, D.N., Rai, A.N. (Eds.), Cyanobacteria. Academic Press, pp. 443–458. https://doi.org/10.1016/B978-0-12-814667-5.00022-2.

Zerkle, A.L., Poulton, S.W., Newton, R.J., Mettam, C., Claire, M.W., Bekker, A., Junium, C.K., 2017. Onset of the aerobic nitrogen cycle during the Great Oxidation Event. Nature. 542 (7642), 465–467. https://doi.org/10.1038/nature20826.