Alkaloids from Cyanobacteria with Diverse Powerful Bioactivities

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Abstract: Alkaloid containing plants represent a heterogeneous group both taxonomically and chemically, a basic nitrogen being the unifying factor for the various classes. As most alkaloids are extremely toxic, organisms containing them do not feature strongly in medicine but they have always been important in the allopathic system. Typical alkaloids are derived from plant sources, they are basic, they contain one or more nitrogen, and they usually have marked physiological actions in humans or other mammalian species.

This review will present various alkaloids generated by cyanobacteria, highlighting their complex structures, powerful bioactivities, and pharmacological properties. The main groups of cyanobacterial alkaloids include the neuromuscular transmission blocker anatoxins, the ion channel blocker saxitoxins, the degenerated amino acid β-methylamino-L-alanine, the protein synthesis inhibitor guanidine alkaloid cylindrospermopsins, and cyanobacterial indol alkaloids with antiviral, antifungal, and cytotoxic activity.

Keywords: Cyanobacterial alkaloids, saxitoxin, anatoxin-A, cylindrospermopsin, indole alkaloids.

INTRODUCTION

The name "alkaloid" in the beginning of its history was originally defined in terms of biogenetic origin and chemical as well as pharmacological activity, however, its current use has changed with time so presently the definition is based on structural properties [1]. The term alkaloid is poorly defined and the distinction between true alkaloids, pseudoalkaloids, and protoalkaloids is often difficult to apply. The modern definition of alkaloid was proposed by Pelletier: an alkaloid is a compound containing nitrogen at a negative oxidation level characterized by a limited distribution in nature [2]. There is no doubt that the first isolation of alkaloids (narcotine, opium, emetine, quinine) in the nineteenth century was an essential step in the evolution of medicine, so the pharmaceutical companies have used and will certainly continue to use alkaloids as biological tools for development of new drugs [3,4].

The genes for the biosynthesis of alkaloids are widely distributed in bacteria, fungi, higher plants, many marine animals (sponges, slugs, worms, bryozoa), arthropods, amphibians (toads, frogs, salamanders), and also in a few of birds and mammals [1,2]. Plants are masters of chemical defence, they are capable to produce special compounds with high diversity called secondary metabolites or allelochemicals [5]. Among these secondary metabolites, alkaloids represent a prominent class of defensive compounds. Over 21 000 alkaloids have been identified so far, which thus constitute the largest group of nitrogen-containing secondary metabolites [6]. Since an alkaloid never occurs alone, alkaloids are usually present as a mixture of a few major and several minor alkaloids of a particular biosynthetic unit, which differ only in functional groups. Secondary metabolites are usually multifunctional. In many cases, even a single alkaloid can exhibit more than one biological function [7]. During evolution, the chemical structure of alkaloids has been shaped so as they usually contain more than one active functional group allowing them to interact with several molecular targets. They can mimic endogenous ligands, hormones, or substrates [8].

Since some of the alkaloids derive from amino acids (often identical with neurotransmitters), it is not surprising that several alkaloids have structural similarities to neurotransmitters. Alkaloids that structurally mimic neurotransmitters can bind to the respective neural receptor. Additional important targets are ion channels, such as the Na^+, K^+, and Ca^{2+} channels [9]. A cytotoxic effect can be produced by several alkaloids. DNA can also be a target: planar and lipophilic alkaloids, such as berberine and sanguinarine, are intercalating compounds that assemble between the stacks of paired nucleotides in the double helix of DNA. Some alkaloids, such as pyrrolizidine alkaloids, aristolochic acids, cycasin alkaloids, are known to form covalent adducts with DNA bases. Ribosomal protein biosynthesis is also often inhibited by alkaloids that interact with nucleic acids [8, 10-12].

Cyanobacteria, the most ancient organisms on earth, have a long evolutionary history [13]. Cyanobacteria are found in several environments from fresh and marine water to rock and soil, including the atmosphere around the earth [14]. These organisms can occur as a single cell or huge mass productions at diverse habitats. During the evolution cyanobacteria have developed several strategies of adaptation to

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extreme environments [13]. Beyond the usual strategies (photosynthesis, fixation of nitrogen, etc.) cyanobacteria produce a great number of specific metabolites of low molecular weight, like peptides, polyketids, alkaloids, and lypopolysaccharides, which all are known to exert strong biological activities [15-17]. In addition, since the chloroplasts of higher plants are supposed to be successors of a cyanobacterial cell, higher plants inherited a number of genes that encode enzymes for pathways leading to secondary metabolites [5].

The ever-larger crowds of cyanobacterial mass appearance and the vigorous development of analytical methods over the past decades have drawn attention to these specific metabolites - now cyanobacteria can be considered as a source for metabolites with great potential for clinical use [18]. In the recent years, several publications dealt with the possible application of the cyanobacterial cyclic and linear peptides as medicines. This review will present various groups of alkaloids produced by cyanobacterial species highlighting their powerful bioactivities, structures, and pharmacological properties.

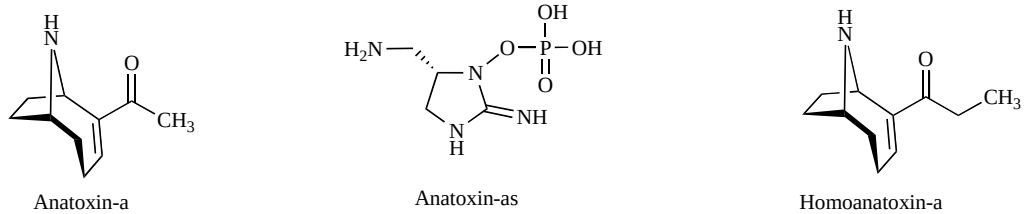
ANATOXINS

Isolation and characterization of these alkaloids began in the early 1960s [19]. These molecules are composed of the structurally related alkaloids anatoxin-a, homoanatoxin-a, and the phosphate ester of a cyclic N-hydroxyguanidine, anatoxin-a(s) (Fig. **1**). Anatoxin-a, a naturally occurring homotropane alkaloid produced by freshwater cyanobacteria of the genera Anabaena, was the first cyanotoxin to have its structure and absolute stereochemistry elucidated. It was originally isolated from *Anabaena flos-aquae* [20, 21]. The molecule is an asymmetrical bicyclic secondary amine being the first naturally occurring alkaloid discovered to contain a 9-azabicyclo[4,2,1]nonane (homotropane) skeleton. Homotropanes are analogues of the tropanes structurally closely related to cocaine [21]. Anatoxin-a is an extremely potent agonist of the nicotinic acetylcholine receptor mimicking the effect of the neurotransmitter acetylcholine (ACh). Anatoxin-a is a depolarizing agent having higher affinity to the receptor than its natural agonist [22]. In contrast to ACh, anatoxin-a is not a substrate of acetylcholinesterase, therefore this alkaloid causes paralysis of the respiratory and other skeletal muscles [16,23].

Anatoxin-a has been a key biomolecular tool for exploring structural features of the nicotinic ACh receptors. Because of the inability of acetylcholinesterase to degrade anatoxin-a and its analogues, these compounds can be used to mimic the effect of ACh when studying kinetic properties of nicotinic ACh receptor function. The natural enantiomer binds to the α4β2 nicotinic receptor with high affinity (K_i = 34 nM) as compared to α3β4 and a7 nicotinic receptors (K_i = 2.5 and 91 nM, respectively) [24]. Successful syntheses of analogues specific to neuronal subtypes could be of great value in the treatment of various neurological dysfunctions. The unique structure of anatoxin-a, and its powerful activity as a pharmacological tool, inspired a multitude of chemical synthetic pathways for anatoxin-a and its analogues [25]. The naturally occurring enantiomer, (+)-anatoxin-a, is much more potent than (-)- anatoxin-a [26]. Under conditions when rat brain α4β2 nicotinic ACh receptors were preferentially labelled with [3H]nicotine, the natural enantiomer was found to be 1000-fold more potent than (-)-anatoxin [27]. Due to its enantiospecificity, anatoxin-a is an excellent tool for probing the stereospecificity of the acetylcholine binding site of nicotinic ACh receptors. Comparing to the natural ligand ACh, the conformation of anatoxin-a is relatively rigid containing only one rotatable bond in contrast to the four ones found in the ACh molecule. Several generations of epibatadine/anatoxin-a homologues have been synthesized and used to probe the structure-activity relationship of subtypes of neuronal α4β2 and α3β4 ACh receptors [28, 29].

Homoanatoxin-a, originally isolated from *Planktothrix sp.* and obtained from various freshwater cyanobacteria, is a relatively rare natural analogue of anatoxin-a in which the C-11 side-chain is extended by one methylene unit. It has recently been isolated from *Raphidiopsis mediterranea* Skuja in Japan and from *Planktothrix formosa* blooms in Ireland [30-32]. Although not as potent as anatoxin-a, homoanatoxin-a was applied in valuable structure-activity relationship studies of anatoxin-a and its homologues [33].

Anatoxin-a(s) is produced in *Anabaena flos-aquae* and *Anabaena lemmermannii* [34]. The symptoms of anatoxin-a(s) intoxication are similar to those of anatoxin-a, but cause increased salivation in vertebrates (as indicated by the index "s"). Anatoxin-a(s) is a naturally occurring organophosphate that is similar in structure to synthetically produced organophosphate-based insecticides [16]. Although organophosphate-based acetylcholinesterase inhibitors have been found in various terrestrial bacteria, such as *Streptomyces antibioticus* [35], anatoxin-a(s) is the only known example produced by cyanobacteria. Anatoxin-a(s) inhibits acetylcholinesterase by acting as an irreversible active-site directed inhibitor preventing degradation of ACh and leading to overstimulation of muscles [36,37]. Thus, although the mechanism of action of anatoxin-a(s) is quite different from that of anatoxin-a, the observed toxicity is similar. Anatoxin-a(s) was the first irreversible acetylcholinesterase inhibitor found in cyanobacteria.

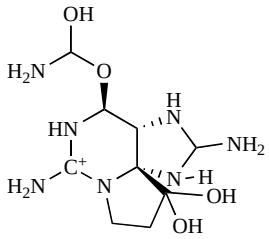


Anatoxin-a Anatoxin-as Homoanatoxin-a

Fig. (1). Structure of anatoxin-a, anatoxin-a(s), and homoanatoxin, all potent cyanobacterial neurotoxins.

SAXITOXINS

Saxitoxin (STX, Fig. **2**) poisoning was observed during dinoflagellate and cyanobacterial blooms. This intoxication is also commonly called as paralytic shellfish poisoning because of the specific blocking effect of STX on voltage-gated Na^+ channels. There are more than 30 naturally occurring STX analogues [17]. These include various forms of ring hydroxylation and sulphation [38-41]. Purified STX is a white, hygroscopic, water-soluble compound with no ultraviolet absorption above 210 nm. Its dihydrochloride salt tends to be stable in acidic solution but loses activity around pH 7–9 [42]. STX is characterized by two pK values (8.22 and 11.28) corresponding to the 7,8,9- and 1,2,3-guanidium groups, respectively [43]. Neosaxitoxin has an additional value of pK at 6.75 which is related to the N-1- hydroxyl group. Paralytic shellfish toxin is a term recently introduced to distinguish STX and its analogues from the syndrome they cause, paralytic shellfish poisoning. The earliest known event of paralytic shellfish poisoning was observed in the United States when a series of *Aphanizomenon flos-aquae* blooms afflicted rural New Hampshire. STX was suggested to have an unusually substituted tetrahydropurine structure, and it was named "saxitoxin" since it was derived from *Saxidonus giganteus* (Alaskan Butter Clam) [44]. Determination of the molecular structure of STX was difficult owing to its non-crystalline, highly polar, and non-volatile nature. Finally, the precise structure has been determined using X-ray crystallography in 1975 [45]. One of the more prominent features of STX and its analogues is the presence of two positively charged guanidinium groups. Since guanidine is one of the few cations which can effectively substitute for Na^+ in the generation of action potentials, it was suggested that the charged guanidinium group of STX enters the Na^+ channel like Na^+ or guanidine, but that the bulky toxin tightly binds to the channel and effectively prevents the entry of Na^+ [46]. Indeed, the 7,8,9-guanidinium group in STX was identified as the biologically active site [47]. This was accomplished by comparing activities of the 1,2,3-guanidinium group of STX and neosaxitoxin under different pH conditions. The essential difference between these closely related analogues is the hydroxylated N-1 group in neosaxitoxin. By adjusting pH conditions the abundance of the protonated form of one guanidinium group could be controlled as well as the overall charge [48]. This manipulation of the two protonated guanidinium groups allowed the estimation of relative activities, leading to discovery of the 7,8,9-guanidinium group as a key structure for activity. STX is a heterocyclic guanidine, which selectively blocks voltage-gated Na^+ channels in excitable membranes [49]. Its use has led to breakthrough discoveries in the field of ion channel physiology pertaining to ion channel structure and function. Because STX blocks Na^+ channels at low concentrations, it was predicted that the toxin acts at a small number of discrete sites in excitable membranes. This was quantitatively shown using tritium-labelled STX preparations in membrane-binding studies [50, 51]. Similar investigations based on this concept were used to demonstrate the mechanism of selectivity and estimate the density of voltage-gated Na^+ channels in various cells and tissues [51-53]. There is strong evidence indicating that STX-induced lethality is caused by a combination of central and peripheral cardio-respiratory effects. Changes in cardiac output can be attributed to the effect of STX on fast Na^+ channels in cardiac Purkinje fibers and working myocardium. Cardiovascular shock resulting from high doses of STX is due to a combination of vascular hypotension and reduced cardiac output – the latter may likely be a consequence of cardiac arrhtyhmias [54-57]. Although STX has been proposed to be a selective blocker of voltage-gated Na^+ channels, new evidence indicates that STX may also interact with some subtypes of Ca^{2+} and K^+ channels [58]. STX has been found to modify the voltage-sensing mechanism of these channels in a complex manner, quite different from the simple pore-blocking model developed for Na^+ channels [58]. Even more intriguing are the variety of naturally occurring analogues available in the paralytic shellfish toxin family. With over 30 analogues found in the nature and an even larger number available through synthetic means, a multitude of structure-activity relationship investigations are possible. Considering these alkaloids as possible therapeutic agents, there are several patent applications and research reports available to demonstrate the strong analgesic effects of STX in combination with other known anaesthetics. Combination increases the efficacy and potency without increasing toxicity [59-61]. New targets for STX, including the L-type Ca^{2+} channel, demonstrate that STX may have further interactions with ion channels of excitable membranes.



Saxitoxin

Fig. (2). Structure of saxitoxin, a paralytic shellfish toxin.

β-METHYLAMINOALANINE

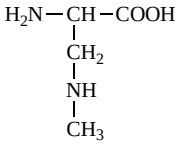
The degenerated amino acid β-Methylamino-L-alanine (BMAA) has originally been found in the seeds of the cycad. The non-proteinogenic amino acid, produced by several cyanobacterial strains (both free living and symbiontic ones), is very similar to the amino acid alanine. BMAA (Fig. **3**) has been proposed as a possible cause of amyotrophic lateral sclerosis [62]. This is a progressive disease of upper and lower motor neurons characterized by weakness and slowed function of skeletal muscles, muscular atrophy and spasticity, tremor, combined with cognitive dysfunctions leading to dementia, symptoms resembling Alzheimer's disease [63, 64]. Testing of various cyanobacterial cultures, potent cyanobacterial symbiontic partners and natural bloom samples showed that several strains that are normally in symbiotic relationship abundantly occurring over the world produce BMAA. BMAA can be biomagnified through the ecosystem. In high-incidence regions focusing on commonalities in customary food, medicinal sources, and revealed plants from the family *Cycadaceae*, BMAA was widely found in all the three groups. BMAA was shown to accumulate in morphologically specialized ''coralloid'' roots of cycas, and more

importantly, the BMAA content of these roots strongly depended on the cyanobacterial partner [65, 66].

BMAA was found in 60-130-fold greater quantity in the protein form than was recovered from the free amino acid pool throughout most of the trophic stages of the Guam ecosystem. It has been suggested that BMAA in protein may function as an endogenous neurotoxic reservoir in humans, and may slowly release the neurotoxin directly into the brain during protein metabolism. Incorporation of a non-proteinaceous amino acid into a protein may have serious impacts, such as creating proteins of aberrant function or occurrence [67]. Furthermore, *in vitro* studies on explanted fetal mouse spinal cord indicate that BMAA may act on glutamate receptors in a manner similar to other excitatory amino acids [68, 69]. Based on its ability to accumulate in cerebral tissue, BMAA may likely be involved in neurodegenerative diseases, therefore, understanding the mechanism of action of this amino acid would be critically important [70].

Of the three subtypes of glutamate receptors, differentiated by their preferred activation by N-methyl-D-aspartate (NMDA), quisqualate, or kainate, β-oxalylaminoalanine appears to operate on those activated by kainite, while BMAA is thought to operate on receptors activated by NMDA [68]. Specifically, β-oxalylaminoalanine was found to be blocked by a broad spectrum of glutamate antagonists but not by NMDA antagonists, while the effects of BMAA are blocked by specific NMDA antagonists [67]. However, BMAA may not be considered as a selective agonist of NMDA receptors, since it was also shown to activate metabotropic glutamate receptors, and also kainate and quisqualate receptors at low concentrations [71, 63]. This BMAA agonism seems chemically incompatible with activity on glutamate receptors, as BMAA lacks the characteristic dicarboxylic acid structure of other excitotoxins [72].

The activity of BMAA is dependent on the presence of extracellular bicarbonate, which may interact with BMAA by forming a carbamate structure. The molecule has a terminal electronegative moiety that is more compatible with the receptor [63]. It was suggested that the overall effects of BMAA may be to depolarize the postsynaptic neurons, thus relieving the magnesium blockade of the Ca^{2+} channel and producing Ca^{2+} influx [73]. This, in turn, may trigger postsynaptic swelling and neuronal degeneration. However, many questions remain to be elucidated regarding the possible role of BMAA in causing amyotrophic lateral sclerosis.

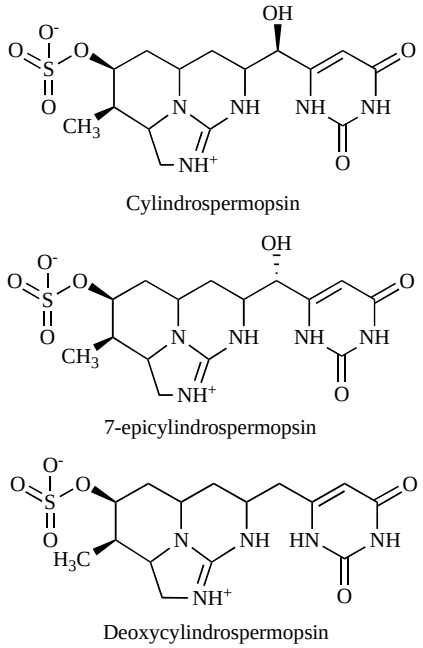


Methylamino-L-alanine

Fig. (3). Structure of β-methylamino-L-alanine, isolated from cyanobacteria.

CYLINDROSPERMOPSIN

Cylindrospermopsin is a naturally occurring alkaloid toxin produced by particular strains of *Cylindrospermopsis raciborskii, Umezakia natans, Aphanizomenon ovalisporum, Anabaena bergii,* and *Raphidiopsis curvata* [74]. The chemical structure of cylindrospermopsin was elucidated in 1992. It consists of a tricyclic guanidine moiety combined with hydroxymethyluracil [75, 76]. Cylindrospermopsin is a dipolar ion with localized positive and negative charges, called zwitterion [76]. Deoxycylindro-spermopsin, an analogue of cylindrospermopsin in which the hydroxyl group on the uracil bridge was removed, has been isolated from *C. raciborskii* and *R. curvata* [77, 78]. Another structural variant of cylindrospermopsin, 7-epicylindrospermopsin, was isolated from *A. ovalisporum* [81]. Structures of these cylindrospermopsin analogues are presented in Fig. (**4**), and the biosynthetic pathway of cylindrospermopsin in Fig. (**5**).



Cylindrospermopsin

7-epicylindrospermopsin

Deoxycylindrospermopsin

Fig. (4). Structure of cylindrospermopsin and its natural derivatives.

Cylindrospermopsin is a white powder that is highly soluble in water. The molecule is chemically stable at sunlight, high temperatures, and a wide range of pH [80]. Based on the nucleoside structure and potentially reactive guanidine and sulphate groups of cylindrospermopsin, it has been speculated that cylindrospermopsin may exert its effects *via* pathways that include reactions with DNA and/or RNA [75, 81]. Covalent binding between DNA and cylindrospermopsin was demonstrated in murine liver *in vivo* [83]. DNA adducts were detected, but not identified, using the ^{32}P-postlabeling assay. This involved extraction of the DNA, hydrolysis into individual nucleotides, labelling of nucleotides using ^{32}P-ATP, separation of adducted nucleotides using two-dimensional thin layer chromatography, and visualization of adduct spots by autoradiography. Cylindrospermopsin also induced DNA strand breakage in murine liver *in vivo*, and increased the number of micronuclei in binucleated cells of the WIL2-NS lymphoblastoid cell-line [75, 81]. Two mechanisms have been suggested for causing the cytogenetic damage: one at the level of DNA to induce strand breaks, and the other at the level of kinetochore/spindle function to induce loss of whole chromosomes [75, 81]. The broad-

spectrum CYP450 inhibitors omeprazole and SKF525A inhibited cylindrospermopsin-induced DNA damage in primary cultured murine hepatocytes at subcytotoxic concentrations, suggesting that CYP-derived metabolites are responsible for cylindrospermopsin genotoxicity, and that genotoxicity is a primary effect of the compound [82].

Natural cylindrospermopsin, synthetic racemic cylindrospermopsin, and selected synthetically-produced cylindrospermopsin structural analogues were assessed for effects on protein synthesis in both rabbit reticulocyte lysate system and cultured rat hepatocytes [84]. No significant differences were observed in the inhibition of protein synthesis elicited by natural cylindrospermopsin and its diol analogue, indicating that the sulphate group might not be essential for the cylindrospermopsin-induced inhibition of protein synthesis. In addition, the orientation of the hydroxyl group at C7 in the carbon bridge does not appear to be important either, since the C7 epimer of cylindrospermopsin (and its corresponding diol) exhibited protein synthesis inhibition similar to that observed with the synthetic racemic cylindrospermopsin. In contrast, the cyclopentyl ring and the methyl and hydroxyl groups on the adjacent hexyl ring may be important structural features, because an analogue lacking these features was 500-1000-fold less potent inhibitor of protein synthesis. The uracil portion of cylindrospermopsin also appears to play an important role in cylindrospermopsin toxicity. It was found that the acute lethality of cylindrospermopsin to mice was eliminated by chlorination or partial cleavage of the uracil moiety (resulting in 5-chloro-cylindrospermopsin and cylindrospermic acid, respectively), as indicated by the comparison of the LD_{50} values obtained for cylindrospermopsin, 5-chloro-cylindrospermopsin, and cylindrospermic acid (0.2 mg/kg, >10 mg/kg, and >10 mg/kg, respectively) [79].

Deoxycylindrospermopsin, an analogue of cylindrospermopsin isolated and purified from *C. raciborskii*, was tested for toxicity in male white Quackenbush mice [78]. Deoxycylindrospermopsin failed to be toxic during 5 days following administration of a 0.8 mg/kg dose i.p., whereas an LD_{50} value of 0.2 mg/kg was reported for cylindrospermopsin in male CD3 mice [76]. Although this comparison suggests that deoxycylindrospermopsin is significantly less toxic than cylindrospermopsin, differences in study designs (e.g., the use of different strains of mice) could have contributed to differences in toxicity.

INDOLE ALKALOIDS

Although a number of indole alkaloids have been isolated from cyanobacteria, these components are produced mainly in higher plants. The biological activity of strychnine and brucin has long been known [85]. This section provides an overview of cyanobacterial indole alkaloids, like bauerines, norharmane, hapalindoles, fischerindoles, welwitindolinones, tjipanazoles, calothrixin, polyconjugated indole pigments, and nostocarboline (Fig. **6**).

Bauerines were isolated and characterised from the terrestrial cyanobacterium *Dichotrix baueriana* GO-25-5. These compounds have strong antiviral activity tested on Herpes simplex virus 2. The most cytotoxic derivative was bauerine C with an IC_{50} of 30 ng/ml. Bauerine A had an IC_{90}

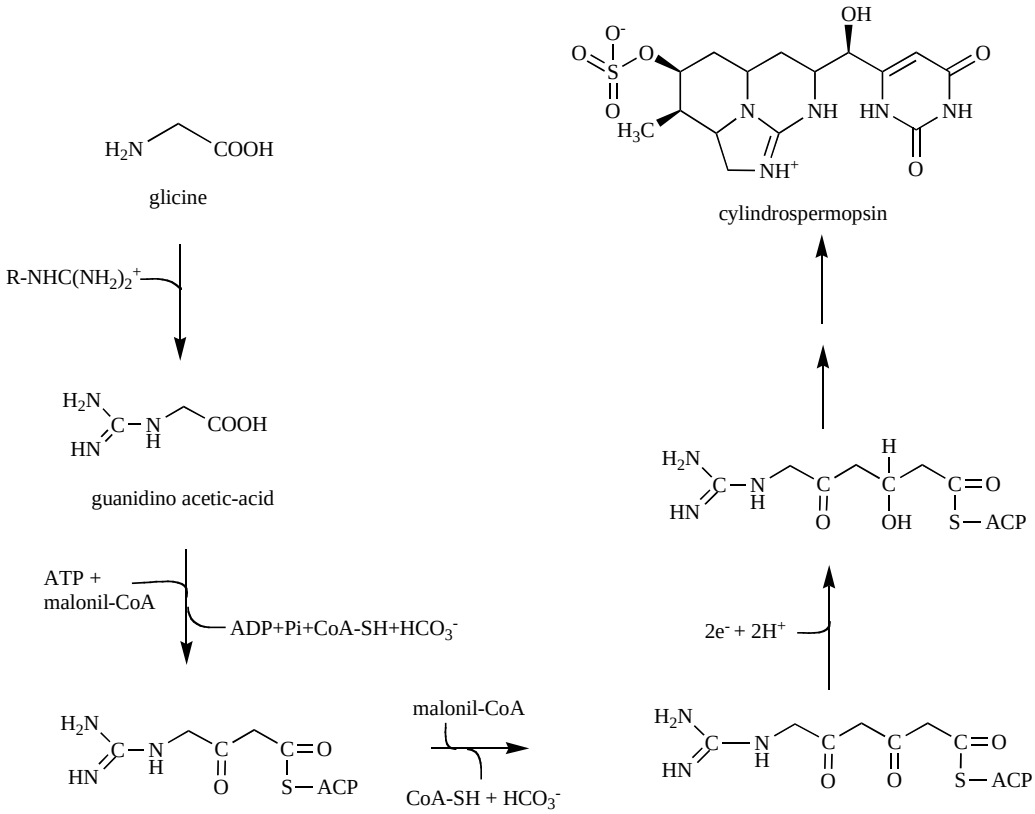
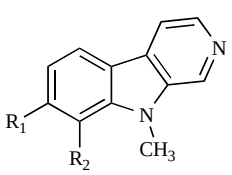
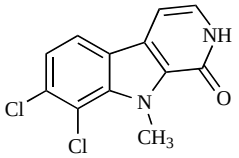


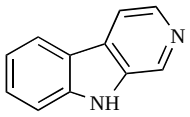
Fig. (5). Biosynthetic pathway of cylindrospermopsin.



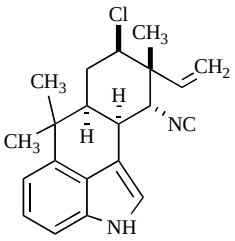
Bauerine A: R1=Cl; R2=H
Bauerine B: R1=R2=Cl



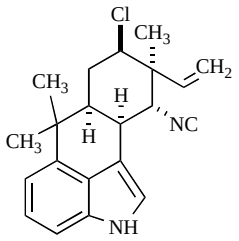
Bauerine C



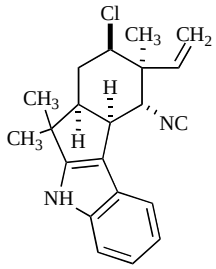
Norharmane



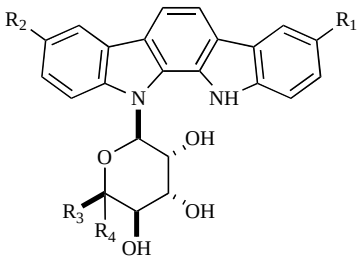
Hapalindole A



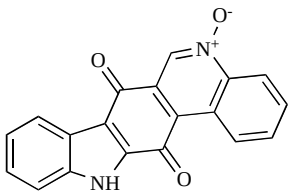
Hapalindole L



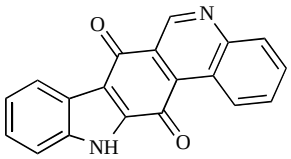
Fischerindole L



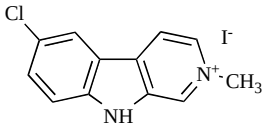
Tjapanazole	R1	R2	R3	R4
A1	Cl	Cl	Me	H
A2	Cl	Cl	H	Me
C1	Cl	H	Me	H
C2	H	Cl	Me	H
C3	Cl	H	H	Me
C4	H	Cl	H	Me
G1	H	H	Me	H
G2	H	H	H	Me



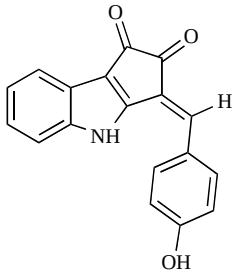
Calothrixin A



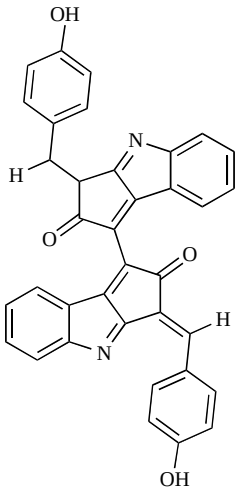
Calothrixin B



Nostocarboline



Nostodione



Scytonemin

Fig. (6). Structure of indole alkaloids, isolated from cyanobacteria.

of 2 μg/ml and cytotoxicity of 3 μg/ml, while bauerine B was found to be less active [86].

Norharmane was isolated from *Nodularia harveyana* in 2005. This indol alkaloid is a well-known molecule in higher

plants with marked antimicrobial activity. The plant hormone auxin (indolo acetic acid) was also identified in cyanobacteria [87].

Several hapalindole derivatives, isolated and characterized from cyanobacterial *Hapalosiphon fontinalis* strains, exert antifungal activity. These metabolites contain a tetracyclic structure with isonitrile and chlorine substituents. All structures show the indole ring connected to a monoterpene unit, mainly with structural variation on the cyclohexane ring. Hapalindole shows structural similarities to lysergic acid, a precursor to ergot alkaloids. The complex derivative of hapalindole, named ambiguine isonitrile, contains a densely functionalized hexacyclic framework. Prenylation of the C(2) of hapalindole followed by ring closure and oxidation results in ambiguine isonitrile D. All these complex indole metabolites display fungicidal activities in a soft-agar disc-diffusion assay. Fischerindole L, isolated from the terrestrial cyanobacteria *Fischerella musciola*, can be considered isomeric to hapalindole A. This metabolite together with derivatives of the hapalindole series, the welwitindolinones, isolated from *Hapalosiphon weilwitschi* (welwitindoline A isonitrile, *N*-methylwitindolinone C isothiocyanate) could be great biological tools as antimicrobial agents [88-93].

Important indole-derived metabolites are the tjipanazoles, isolated from *Tolypothrix tjipanasensis* [94]. The biological activity of these compounds is characterized by antifungal properties, and show weak activity against leukaemia and solid tumor cell lines without inhibition of protein kinase C up to the concentration of 1 μM. In a screening program of cyanobacterial metabolites against *Plasmodium falciparum* two new indoloquinones, calothrixin A and B, were isolated and their structure determined. These compounds share the same indolo[2,3-a]carbazole framework as other bioactive compounds, such as staurosporine, rebeccamycin, or the synthetic enzastaurin. Tjipanazoles generally lack the pyrrolo[3,4-c] ring, their indolo[2,3-a]carbazole scaffold can be chlorinated at various degrees and glycosylated with 6-deoxy-D-gulose (tjipanazoles A1, C1, C2 and G1) and L-rhamnose (tjipanazoles A2, C3, C4 and G2). These compounds contain an indolo[3,2-j]phenanthridine skeleton, which is unprecedented in other natural products. They display nanomolar activity against *P. falciparum* with IC_{50} values around 100 nM. Calothrixin B was found to be less active than the *N*-oxide derivative (IC_{50} of 58 *versus* 180 nM), but still retained submicromolar activity. The well-known antimalarial agent chloroquine displayed an IC_{50} of 83 nM. These indoloquinoline metabolites also inhibit the growth of HeLa cancer cell lines, an IC_{50} of 40 nM was obtained for calothrixin A [95-102].

The carbolinium alkaloid nostocarboline was isolated and its structure characterized from *Nostoc* 78-12A. This compound contains a chlorine atom, which is unusual for a freshwater metabolite. Nostocarboline is an inhibitor of cholinesterase, an enzyme targeted in the treatment of Alzheimer's disease [103, 104].

Because of the photoautilitothrophic mode of these organisms, cyanobacteria populate the habitats exposed directly or indirectly to solar radiation. At some habitats like deserts, stones and water surface, this requirement results in their exposure not only to the visible part of the spectrum but also to the shorter wavelengths. The use of UVR-absorbing compounds as sunscreens is known in many organisms, as a passive mechanism for protection. Extracellular sheath pigments with high chemical diversity have been observed in some terrestrial forms of cyanobacteria [13]. Cyanobacteria produce polyconjugated indole pigments as sunscreens. One of these pigments, scytonemin has been known for more than 100 years, although its chemical structure was characterized only in 1993 [105]. It contains a dimeric structure probably derived from tryptophan and polyketide biosynthetic pathways. Later, a postulated intermediate, nostodione was isolated from a *Nostoc* species [106]. Nostodione, which was obtained by ozonolysis of scytonemin, could be reductively dimerized to the dimeric parent structure, scytonemin [105]. Another indole, prenostodione, was isolated and postulated to serve as precursor to nostodione [107, 108]. However, another possibility of its formation could be oxidation of the dione fragment of nostodione during the isolation procedure. These chemical structures may be used as a model when developing new UVR-protective creams.

Table 1. Bioactivities of Cyanobacterial Alkaloids

Cyanobacterial alkaloid	Activity
anatoxin-a	nicotinic acetylcholine receptor agonist
anatoxin-a(s)	cholinesterase inhibitor
Saxitoxin	voltage-gated sodium channel blocker
β-Methylamino-L-alanine	triggering amyotrophic lateral sclerosis
cylindrospermopsin	protein synthesis blocker
bauerines	antiviral agent
norharmane	antimicrobial agent
hapalindole	antifungal agent
tjipanazoles	antifungal agent
calothrixin A and B	antimalarial agent
nostocarboline	cholinesterase inhibitor
scytonemin	UVR-protective agent

CONCLUSIONS

This review gave an overview of the variety of cyanobacterial alkaloid metabolites. Some of these molecules, like anatoxins and saxitoxins, are strongly toxic, but based on their specific interactions with their natural targets, these compounds are useful tools in physiological and pharmacological research. Some indol alkaloids, produced by cyanobacteria, can be utilized as anti-fungal agents. Nostocarboline, a chlorine containing compound, is a cholinesterase inhibitor with promising perspectives in the treatment of Alzheimer's disease (Table **1**). Beyond these examples, the diverse chemical structures of cyanobacterial alkaloid metabolites combined with strong biological activities may in-

spire both the chemist and the pharmacologist to develop new powerful drugs as a response to the challenges of today.

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ABBREVIATIONS

ACh = Acetylcholine

BMAA = β-Methylamino-L-alanine

NMDA = N-methyl-D-aspartate

STX = Saxitoxin

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