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Secondary Metabolites from Cyanobacteria: Complex Structures and Powerful Bioactivities

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Abstract

This review presents natural products from cyanobacteria. Several classes of secondary metabolites are highlighted. Toxic metabolites from these prokaryotic photosynthetic organisms include compounds such as microcystin, anatoxin and saxitoxin, which display hepatotoxicity and neurotoxicity. Their potential as drugs in cancer therapy is discussed based on the cryptophycin class of potent cytotoxic agents. The next part of this review highlights iron chelators from cyanobacteria, including schizokinen, synechobactin and anachelin. The biogenesis of anachelin is investigated as its mechanism of iron acquisition. Several indole alkaloids are then reviewed, from simple carbolines such as bauerines and nostocarboline to complex polycyclic structures such as hapalindole, welwitindolinone and ambiguine. The latter compounds present fascinating structure combined with powerful bioactivities and interesting biogenetic pathways. In the last part, protease inhibitors from cyanobacteria are discussed (cyanopeptolins, micropeptin and oscillapeptin) and their structure/activity relationships and selectivity for trypsin / chymotrypsin are presented. All these examples highlight the large structural variety of cyanobacterial metabolites combined with powerful biological activities. Cyanobacteria can thus be considered a prime source both for novel bioactive compounds and for leads for drugs.

Keywords

Natural products – Isolation – Structure determination – Biosynthesis – Medicinal chemistry – Total synthesis

1 Introduction

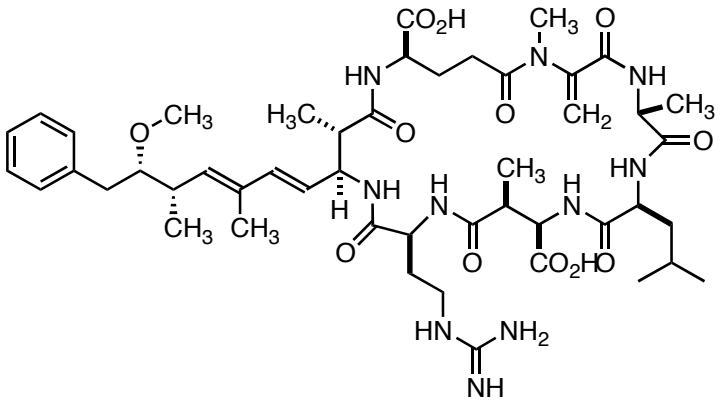
Cyanobacteria are among the most successful and oldest life forms still present on earth. These prokaryotic photoautotrophs populate habitats as diverse as soil, marine and freshwater environments, rocks (endolithic), plants or animals (as endosymbionts) to ice. This vast variety of different ecological niches, in some of which tough competition for nutrients rules, requires successful adaptation to vastly different conditions. In order to adapt to these surroundings, cyanobacteria evolved to produce a wide variety of different secondary metabolites.[1-6] In particular cyanobacteria from marine and freshwater sources produce many metabolites which possess powerful biological activities.[1,3] This review will present different classes of secondary metabolites generated by cyanobacteria, will highlight their complex structures and powerful bioactivities and will discuss their relevance to human health and society.

2 Toxic metabolites from cyanobacteria

The class of cyanobacterial secondary metabolites that recently received wide attention from different scientific communities as well as the general public, are cyanobacterial toxins or cyanotoxins.[5-7] One of the best studied classes of cyanobacterial toxins are the *microcystins*, modified peptides produced by a large variety of different cyanobacteria such as *Anabaena*, *Microcystis*, *Hapalasiphon*, *Nostoc* and *Oscillatoria*.[8-13] The general structure of microcystins is described by *cyclo*(-D-Alal^1-Xaa^2-D-MeAsp^3-Yaa^4-Adda^5-D-Glu^6-Mdha^7-), whereas the amino acids Xaa^2 and Yaa^4 are highly variable and determine the suffix in the nomenclature of microcystins.[14] For example, the microcystin shown in Fig. (**1**) is called Microcystin LR (Xaa^2 = Ala, Yaa^4 = Arg).[10] So far, more than 50 different variants of microcystins have been found, all featuring the β-amino acid (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldecy-4(*E*),6(*E*)-dienoic acid abbreviated as Adda.[9,10] This amino acid was shown to be essential for the biological activity of microcystin *via* the covalent modification of proteins.[10,15,16] Microcystins are highly active against protein phosphatase 1 and 2A.[17,18] These proteins are essential for many signal transduction pathways of eukaryotic cells such as programmed cell death (apoptosis).[19,20] This enzyme inhibitory activity is also responsible for the toxicity of microcystins to grazers and thus point to their ecological role as deterrence chemicals in aquatic ecosystems.[21,22] Recently, microcystins caused great concerns to human health organizations. Due to the known hepatotoxicity of microcystins, elevated levels of liver cancer were related to the presence of microcystin in drinking water.[7] Actually, *Microcystis* is the most prevalent

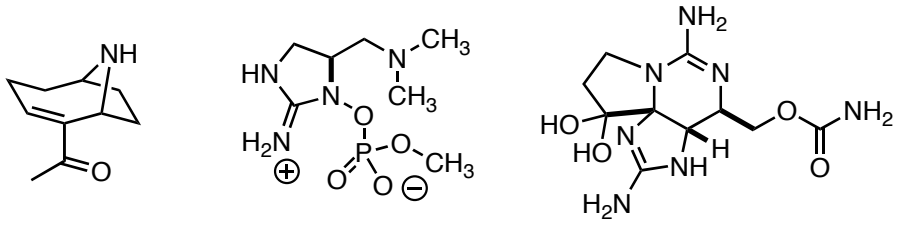
cyanobacterial genus to be suspected with human poisoning.[23] As a consequence, the World Health Organization (WHO) has set threshold values for microcystin concentrations in drinking water.[24]

Fig. (**1**). Microcystin LR, a cyanobacterial toxin from *Microcystis aeruginosa*.



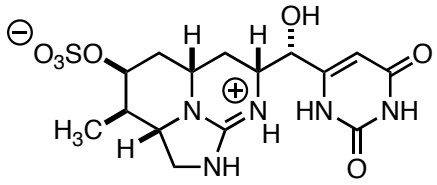
Another class of important cyanobacterial toxins includes alkaloids such as the anatoxin and saxitoxin Fig. (**2**).[4,6,9,11,12] Anatoxins (originally called fast death factors) are neurotoxins and affect the nervous system, the skin (swimmer's itch) or the gastro internal tract.[25-27] In high doses, anatoxins lead to death due to respiratory failure. Whereas anatoxin-a is a bicylic amine, the more complex anatoxin-a(s)[28-31] features an interesting phosphate ester of a cyclic *N*-hydroxyguanidine motif. These compounds act upon acetylcholinesterase[28] and thus lead to toxic effects and ultimately death in higher organisms.[29]

Fig. (**2**). Anatoxin, anatoxin-a(s) and saxitoxin, potent cyanobacterial neurotoxins.



Saxitoxin,[32,33] originally identified from axenic cultures of the dinoflagellate *Gonyaulax catenella*,[34] is also produced by several cyanobacteria.[35] This tricyclic alkaloid, structurally related to tetrodotoxin,[36,37] blocks neuronal transmission by binding to Na channels in nerve cells.[38] The sodium gradient is thus stopped, which leads to muscle paralysis and thus death in mammals.[38] Saxitoxins can be a cause of paralytic shellfish poisoning in humans consuming contaminated shellfish, as these toxins are bioaccumulated by marine molluscs and crustacaens.[39]

Fig. (**3**). Cylindrospermopsin, a toxin from a variety of cyanobacteria

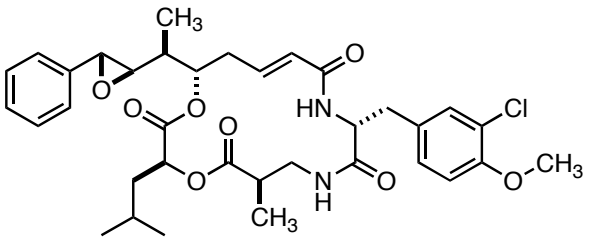


Cylindrospermopsin, isolated from a variety of freshwater cyanobacteria such as *Cylindrospermospsis*, *Anabaena bergii* and others,[40-42] can also be considered a threat to drinking water supplies Fig. (**3**).[43,44] This alkaloid possesses an interesting structure merging an uracil fragment to a tricyclic guanidinium moiety.[41] Poisonings of cattle livestock and even humans have been attributed to the occurrence of toxic *Cylindrospermospsis* blooms in lakes and thus to cylindrospermopsin.[43,44] The mode of action of this alkaloid was proposed to involve DNA intercalation and followed by strand cleavage.[45]

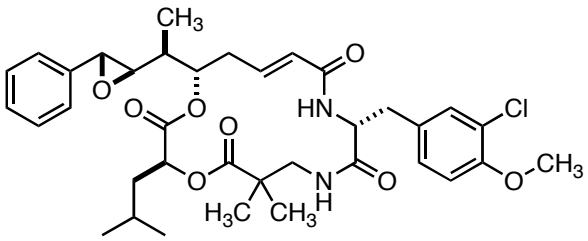
Cyanobacteria have been recently considered a source for metabolites with great potential for clinical use.[46-49] The most prominent member of a cyanobacterial product with potent anticancer properties is cryptophycin, originally isolated as fungicide in 1990 from a *Nostoc* strain Fig. (**4**).[50] Its structure is typical for cyanobacterial metabolites, as it is composed out of a polyketide fragment, a modified D-α-amino acid, a β-amino acid and a hydroxyacid forming a depsipeptide. While cryptophycin was first identified for its potent antifungal activity against *Cryptococci*,[50] it was later abandoned due to its notable toxicity. Later, Moore and coworkers isolated the same compound from another *Nostoc* strain[51] and

determined powerful cytotoxicity against tumor cell lines with IC_{50} value in the order of 10 pM.[52,53] In addition, cryptophycin was not a substrate for the P-glycoprotein efflux pump, as activity was found against both drug-sensitive and drug–resistant tumor cell lines.[53] This cyanobacterial peptide polyketide hybrid induces apoptosis by blocking the cell cycle at the G2/M phase apparently via inhibition of tubulin polymerization.[54] The mode of action of cryptophycin combined with its extreme potency (up to 1000 fold greater than taxol), led to its clinical evaluation as well as structure/activity relationships (SAR).[55]

Fig. (**4**). Cryptophycin 1 and 52, potent antitumor agents isolated from cyanobacteria.



Cryptophycin 1



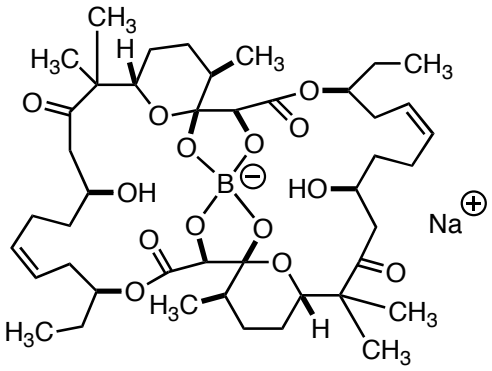
Cryptophycin 52

Over the years, more than 25 different cryptophycins were isolated from natural sources allowing for detailed SAR studies.[55] Interestingly, the most abundant compound (220 mg cryptophycin 1 were isolated from 50 g algae), was the most cytotoxic entity. The epoxide was essential for activity, as were all structural features of the polyketide fragment.[55] Modification of the aromatic ring of the D-amino acid were tolerated, whereas replacement with L-amino acids led to a reduction of activity by five orders of magnitude.[55] In order to complement the SAR

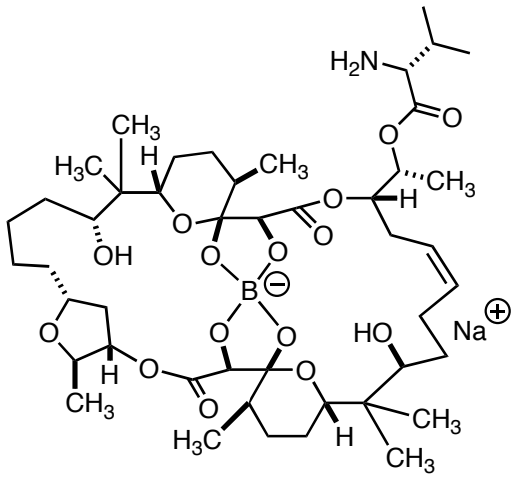
of the natural isolates, researchers both in academia and in industry (Eli Lilly) embarked on the synthesis of hundreds of analogs, allowing for a detailed understanding of the structural requirements for the biological activity of cryptophycins.[55] Cryptophycin 52 was found to be more stable towards hydrolysis and was thus selected as the main candidate for clinical development by Eli Lilly (LY355703).[56,57] While this compound passed phase I clinical studies,[56,57] there is little data in the literature concerning phase II studies.[58]

Borophycin is a complex boron containing polyketide, isolated in 1994 by Moore and coworkers from *Nostoc linckia*[59] and later from *Nostoc spongiaeforme* Fig. (**5**).[60] This metabolite displayed promising antitumor activity against standard cancer cell lines (MIC 0.066 mg/mL, LoVo and 3.3 mg/mL KB).[59] The structure was assigned by spectroscopic methods and both configuration and constitution unambiguously established by single crystal X-Ray diffraction. The structure can be regarded as a dimeric macrodiolide, with a borate linking the two strands. Its structure strongly resembles boromycin from *Streptomyces antibioticus*, isolated in 1969 by Prelog, Zähner, Keller-Schierlein and coworkers[61] and structure determination by Dunitz, Prelog and coworkers,[62] aplasmomycin from *Streptomyces griseus*[63,64] and tartrolon from *Sorangium cellulosum*.[65]

Fig. (**5**). Borophycin from cyanobacteria and boromycin from streptomyces.



Borophycin



Boromycin

Borophycin remains as the only metabolite isolated so far from cyanobacteria serving as a ligand to a semi-metal. In the next chapter, an overview of small cyanobacterial metabolites as ligands for iron will be presented.

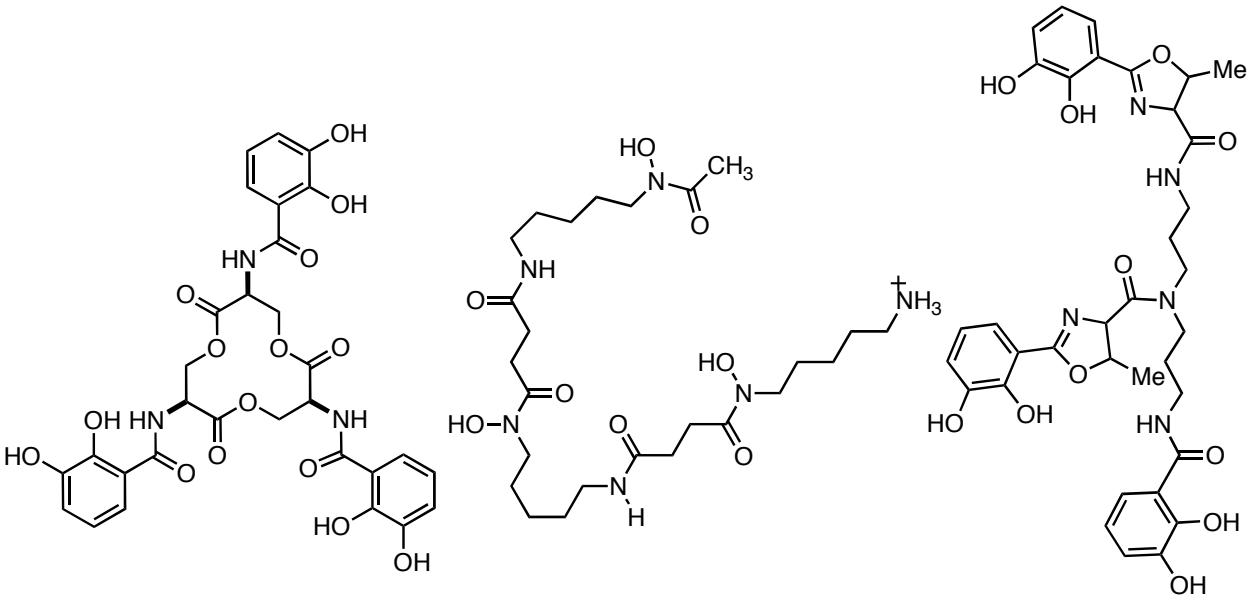
3. Iron chelators from cyanobacteria

Cyanobacteria are considered to be among the oldest organisms on earth, populating this planet since over 2.5 billion years. Over 2 billions years ago, these organisms are thought to release oxygen in earth's atmosphere thus changing from a reductive to an oxidative environment as it is present today.[66,67] This change had dramatic consequences as almost all life forms were forced to adapt to these oxidative conditions or to become extinct. Moreover, the change from a reductive to an oxidative atmosphere had the detrimental side effect that the soluble and

prevalent Fe(II) salts were oxidized to the corresponding Fe(III) oxide hydrates, which are almost insoluble.[68] Due to this extremely low bioavailability of iron, the acquisition, transport and storage of Fe became a central challenge for every organism.[68] Microorganisms thus evolved sophisticated strategies for Fe sequestering involving small organic ligands, so called siderophores.[69,70] In fact, many siderophores from bacteria have been isolated which can be clustered in three different structural types.[69,70]

The first group is the catecholate siderophores exemplified by enterobactin from enterococci Fig. (**6**).[71,72] Its structure is characterized by a C_3 symmetric trilactone ring, of which the exocyclic N atoms are acylated by dihydroxybenzoic acid. These ligands bind Fe with exceptionally high binding constants of up to 10^{47}.[73] The release of iron in the cell is accomplished by protonation of the catecholate, by enzymatic hydrolysis of the lactone ring or by reduction of Fe(III) to Fe(II).[71]

Fig. (**6**). The three main structural classes of bacterial siderophores.



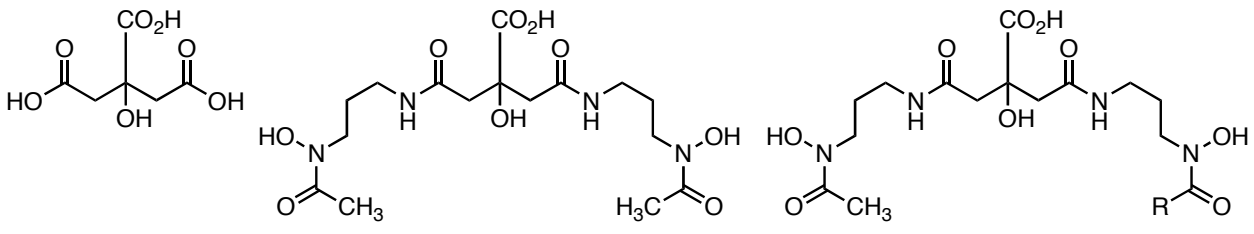
Enterobactin Desferrioxamine B Vibriobacin

Another class of siderophores is represented by hydroxamate siderophores exemplified by desferrioxamine (DFO), isolated by Prelog and collaborators fifty years ago.[74-76] Key elements are hydroxamic acid units which can chelate to Fe(III) in a bidentate way. The binding constants of DFO have been determined to be between 10^{20} and 10^{30}, depending on the structure and on the pH.[77,78] DFO is a drug currently used to treat iron overload.[79] Iron is so precious to humans that men have no means of secreting excess iron out of the body.[80] Excess iron leads to oxidative stress followed by tissue damage and, ultimately, death.[81] Moreover, hemochromatosis is the most prevalent genetic disorder in Europe with 1 out of 20 carrying the mismatched gene, [82] and this disease is directly linked to high iron levels. Iron overload can only be cured by either phlebotomy or treatment with DFO.[83] The drawback of DFO is that it needs to be administered for 6 days for at least 12 hours intravenously due to poor pharmacokinetics. Patient compliance is

thus a significant problem. Novartis has recently introduced a small molecule for treating iron overload (ICL-670).[79]

The last class of iron chelators are the oxazoline/thiazoline catecholates exemplified by vibriobactin from *Vibrio cholerae*[84] or yersiniabactin from Y*ersinia pestis*.[85-87] In recent years, research on these virulence factors surged in order to better understand bacterial infections.[88,89] Moreover, interesting new therapeutic approaches to treat such diseases have been developed based on siderophores. Examples feature the so-called “trojan horses” developed in the research groups of Braun,[90] Miller, [91,92] and Budzikiewicz.[93] The design of these compounds involves the conjugation of an antibiotic fragment to a siderophore, a principle which nature also uses in antibiotics such as ferrimycin,[94,95] albomycin[96-98] or salmycin.[99] As bacteria recognize extracellular siderophores and have developed active mechanisms for their uptake, the antibiotic fragment is transported inside the bacteria. Therefore, these siderophore/antibiotics conjugates are highly active. However, since a key pathway of bacterial survival, *i.e.* iron acquisition, is targeted, resistance to these compounds quickly develops.[100]

Fig. (**7**). Cyanobacterial siderophores derived from citric acid.



Citric acid

Schizokinen

Synechobactin A R = $C_{11}H_{23}$
Synechobactin B R = C_9H_{19}
Synechobactin C R = C_7H_{15}

Whereas bacterial siderophores have been actively studied for decades, there is much less known about cyanobacterial iron chelators. This is surprising, as cyanobacteria probably caused the transformation of a reductive atmosphere to an oxidative one and thus rendered the uptake of Fe(III) a central challenge to every organism.[66,67] Another problem is that cyanobacteria frequently populate marine and freshwater habitats and are among the most important primary producers. In such environments, complex siderophores, which are costly to make by the organism involving many enzymes and pathways), are lost by dilution when secreted. Therefore, it was assumed for a long time that only small and simple organic molecules such as simple hydroxamic acids or citrate would be used. Their benefit would include ease of preparation with the drawback of poorer iron chelation.

The first report on a cyanobacterial siderophore was schizokinen from the freshwater *Anabaena* PCC 7120 Fig. (**7**).[101-103] This compound, originally isolated from the terrestrial bacterium *Bacillus megaterium*,[104,105] is a citrate derivative of which two carboxylate groups are amidated with an acetyl hydroxamic amine. In 2005, the synechobactins were isolated from a coastal marine cyanobacterium, *Synechococcus* PCC 7002 and demonstrated to act as siderophores.[106] These compounds can be considered as derivatives of schizokinen, with one of the hydroxamic acids replaced by a long fatty acid. The authors suggest that this amphiphilic structure helps to anchor the siderophore into the membrane of cyanobacterium and thus prevents its loss by dilution in marine environments. This notion is supported by several amphiphilic siderophores from marine bacteria such as marinobactin E, aquachelin D and amphibactin D, which all carry a long

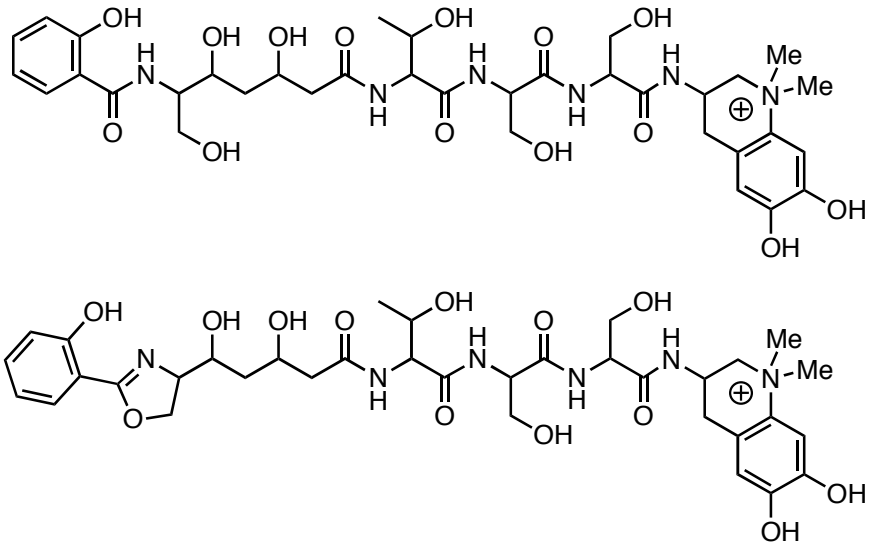
hydrophobic chain.[107] The citrate core of both schizokinen and synechobactin are also found in petrobactin.[108]

The first complex siderophore, and only the second siderophore to be isolated from cyanobacteria were the anachelins, produced by *Anabaena cylindrica* 1403-2a. This blue-green alga, originally isolated in 1939 from a pond in Surrey, is forming filaments that contain heterocysts, where N_2 fixation can occur. The filaments of *A. cylindrica* form biofilms or cyanobacterial mats that are attached to surfaces Fig. (**8**). Upon longer growth and concomitant oxygen production, the cyanobacterial mats detach from the surface forming a cyanobacterial bloom.

Fig. (**8**)**.** A culture of *Anabaena cylindrica* 1403-2a showing how this filamentous cyanobacterium forms biofilms attached to surfaces.

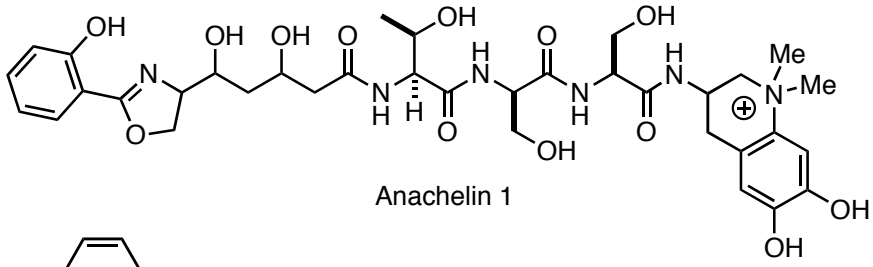
Two anachelins were isolated from this *Anabaena* by Budzikiewicz, Walsby and coworkers and reported in 2000: Anachelin H containing a terminal salicylamide and anachelin 1, terminated by an oxazoline ring Fig. (**9**).[109] These compounds are structurally complex metabolites, which contain fragments of different biosynthetic origin. The salicylate unit, which can emanate out of a polyketide pathway or be derived from the chorismate pathway, a mixed polyketide/amino acid fragment, a tripeptide part and an unusual tetrahydroquinolinium alkaloid fragment. Thus, anachelin can be considered natural product hybrid. [110] While the constitution was published, it was not possible for this group to determine the absolute or relative configuration of any of the stereogenic centers.

Fig. (**9**). Anachelin H and anachelin 1, isolated from *Anabaena cylindrica* 1403-2a.

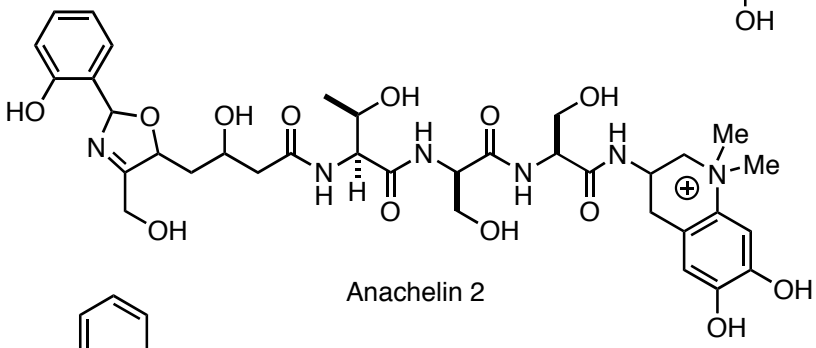


Subsequently, Murakami and coworkers reported the isolation and structure elucidation of anachelin 1 and additional isomers Fig. (**10**).[111] In addition to the terminal oxazoline, the authors also obtained anachelin 2 featuring the oxazoline ring from C(5)-O to the C(6)-N. Moreover, two related esters were obtained. The authors were also successful in determining the absolute configuration of the tripeptide fragment thus establishing the sequence as L-Thr-D-Ser-L-Ser. The configuration of the polyketide fragment as well as the alkaloid part remained unassigned.

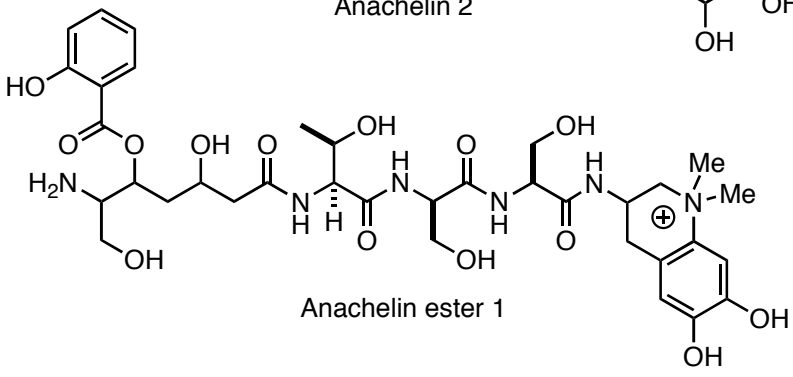
Fig. (**10**). Anachelin 1, anachelin 2 and related esters.



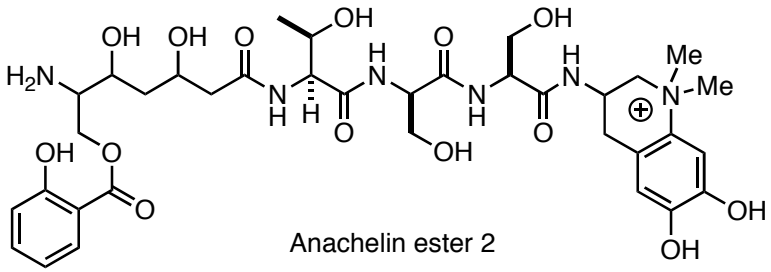
Anachelin 1



Anachelin 2



Anachelin ester 1

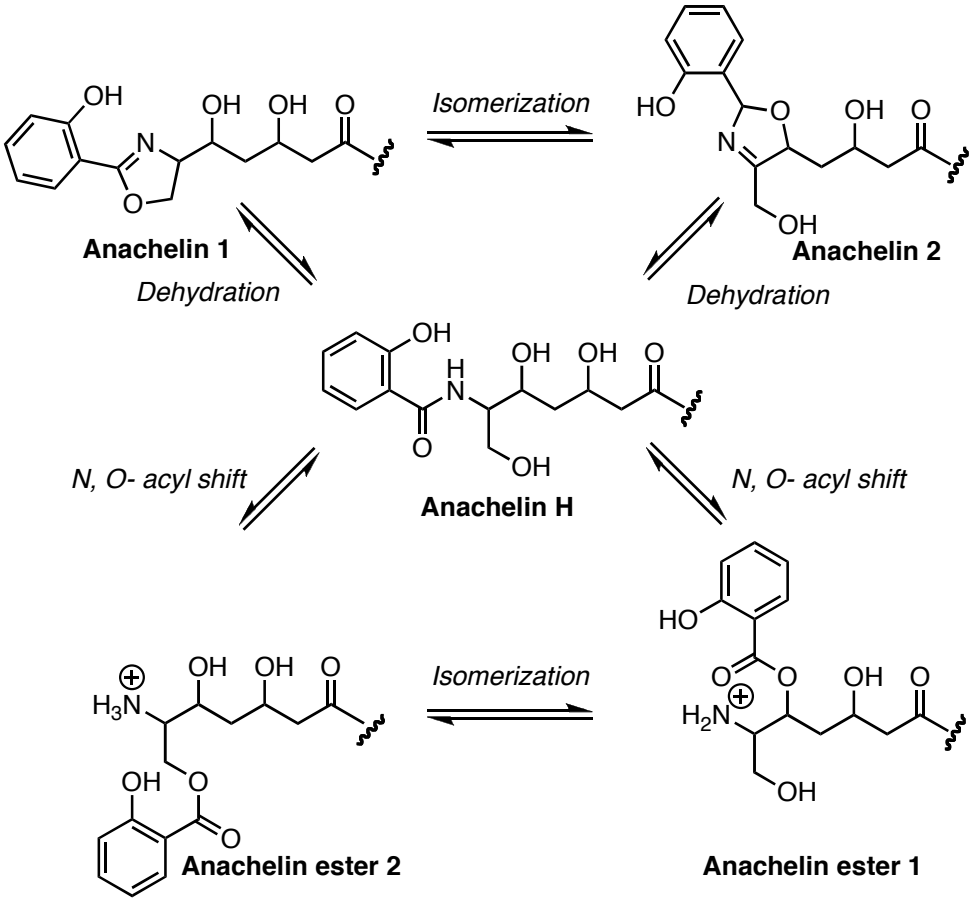


Anachelin ester 2

Out of the five anachelins isolated, the question can be raised which of the compounds are present in nature and which compounds could be isolation artifacts. Budzikiewicz, Walsby and coworkers always obtained mixtures of anachelin H and anachelin 1, and were never able to isolation anachelin 1 in pure form.[109] Moreover,

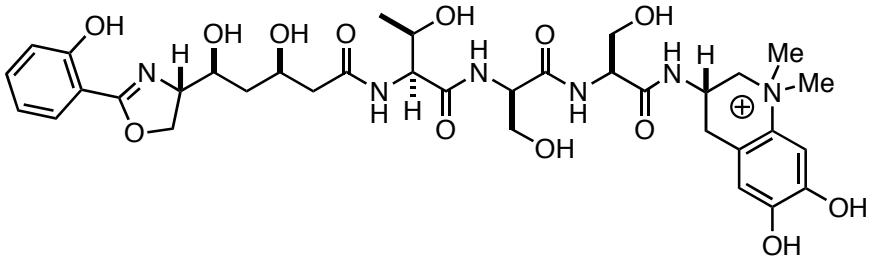
these authors stated that the oxazoline ring in anachelin 1 is prone to hydrolysis upon standing in aqueous solution.[109] This experimental evidence thus suggests that the biologically active compound is anachelin H, and that the oxazoline of anachelin 1 and anachelin 2 are formed upon dehydrating conditions. This could be the case when extracts are purified by using HPLC with trifluoracetic acid/acetonitrile/water mixtures. The lyophilization of fractions containing anachelin H could lead to acid catalyzed dehydration Fig. (**11**) top.

Fig. (**11**). Possible pathways for the formation of anachelin 1, anachelin 2 and the related esters from anachelin H.



The formation of the esters could be easily explained by an *N*, *O*-acyl shift of the salicylamide under slightly acidic conditions. This shift would be favored due to the protonation of the amino group in acidic medium. This would provide an explanation why Murakami and coworkers did not obtain anachelin H, but instead the isomeric esters.[111] *N, O*-acyl shifts are frequently observed in peptides and a well-known example is the formation of iso-cyclosporin derived from cyclosporin via an *N, O*-acyl shift of the MeBmt amino acid.[112-116] This migration readily occurs under acidic conditions and is fully reversible upon the addition of base.[116] Last, anachelin is the siderophore of an aquatic freshwater siderophore, which should be resistant to facile hydrolysis. This requirement adds further support to the role of anachelin H, as anachelin 1 was found to be prone to hydrolysis. All these reasons provide support for the hypothesis that anachelin H constitutes the actual siderophore, and that anachelin 1 and 2, as well as the related esters would be considered derivatives.

Fig. (**12**). The absolute configuration of anachelin 1 as determined by Murakami and coworkers.[117]



In the initial isolation report, Murakami and coworkers were not able to determine the absolute configuration of the polyketide and alkaloid fragments.[111] In a subsequent publication, Murakami and coworkers established the relative and

absolute configuration of anachelin via degradation and spectroscopic analysis and published their analysis in 2004 Fig. (**12**).[117] We have independently established the relative and absolution configuration via a stereodivergent synthesis. [118]

The tetrahydroquinoliunium chromophore of anachelin is highly unusual, and its biogenesis is unknown. We have postulated a biogenetic hypothesis Fig. (**13**), [119] that was used as a blueprint for a total synthesis of this metabolite. [118] We hypothesized that the unusual chromophore is biogenetically derived from a C-terminal tyrosine. Reductive amination and methylation would give the dimethyltyrosinamine precursor. Hydroxylation followed by oxidation would lead to the *ortho* quinone that can react in an intramolecular aza annulation reaction that would furnish, after tautomerization, the anachelin chromophore. In order to test this hypothesis in the chemical laboratory, and to evaluate potential enzyme catalysts of this transformation, we prepared the model precursor as shown in Fig. (**14**).

Fig. (**13**). Biogenetic proposal for the formation of the anachelin chromophore.

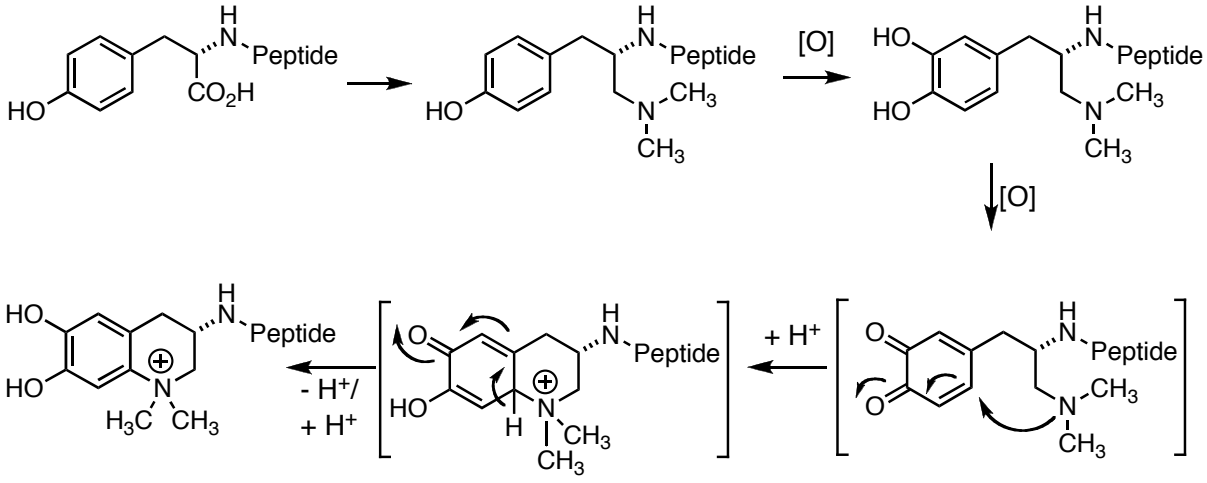
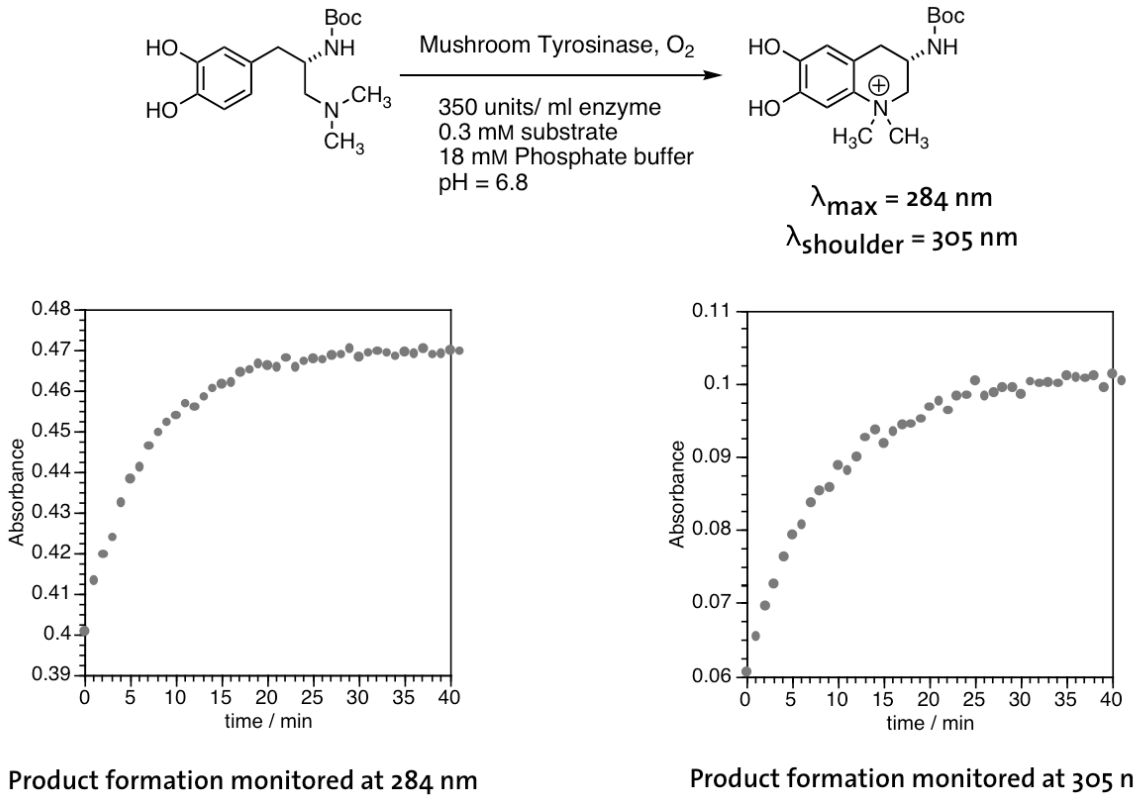


Fig. (**14**). Biomimetic formation of the anachelin chromophore by the enzyme tyrosinase.



Exposure of this diamine to tyrosinase, an ubiquitous enzyme involved in tyrosine and DOPA oxidation, gave rise to the anachelin chromophore. The formation of this compound is observed spectroscopically within minutes, and the product anachelin chromophore could be isolated and characterized on a preparative scale. These experiments provide a clear rationale for the involvement of this enzyme in the biogenesis of the anachelins. [120]

Our investigations on the solution structure,[121] biogenesis,[120] synthesis,[118,119] and mode of action[122] of anachelin led to a thorough molecular understanding of the

different facets of this compound. This knowledge also allowed for the application in a totally different field, *i.e.* surface modification in materials science. The predominant physical form of iron in marine and freshwater environments are iron oxide hydrates, which are solid minerals. In addition, iron ions are present in stones, rocks and minerals. Therefore, in order to apprehend iron ions, siderophores must recognize, bind and sequester iron from solid minerals. The first step of this process in binding of the siderophore to mineral surfaces. We therefore asked the question whether it is possible that anachelin binds to metal oxide surfaces. In order to test this hypothesis, we prepared the hybrid[110] of the anachelin chromophore and polyethylene glycol (PEG), which is frequently used to render surfaces resistant to the attachment of proteins. The resulting compound was clearly shown to efficiently bind to titanium oxide surfaces.[123] Thus, it has been shown that the anachelin chromophore recognizes metal oxide surfaces as found in minerals. This postulated first step in iron acquisition is thought to have large implications on the molecular understanding of iron acquisition of marine and freshwater organisms.

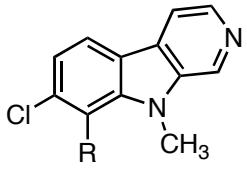
4 Indole alkaloids

Indole alkaloids are among the most frequently encountered classes of secondary metabolites in higher plants, and to a lesser extent, in microorganisms and animals.[124] Prominent examples include the *ergot*, *iboga*, *pyrroloindole* (*i. e.* physiostigmin), *carboline* (*i. e.* harmane, reserpine, yohimbin), *aspidosperma* and the *strychnos* alkaloids (*i. e.* strychin, brucin and curare).[124] In most of these cases, potent biological activities are associated with this structural framework, and the

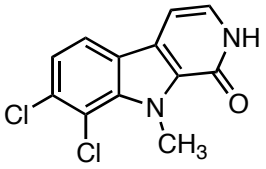
isolation and structure elucidation of these compounds was of fundamental importance for the understanding of their mode of action for the development of related substances with beneficial effects.

In contrast to this wealth of indole alkaloids from higher plants, there are much less cyanobacterial indole metabolites known so far. Larsen, Moore and Patterson of the University of Hawaii isolated the bauerines A-C from the terrestrial cyanobacterium *Dichotrix baueriana* GO-25-5 and determined their structure Fig. (**15**).[125] These compounds were found in the context of a screening program of hundreds of cyanophytes against Herpes simplex virus 2 (HSV-2), followed by bioassay-guided fractionation. While bauerine A was found to have an IC_{90} against HSV-2 of 2 μg/mL with a cytotoxicity of 3 μg/mL (LoVo), bauerine B was found to be less active. The most cytotoxic derivative was bauerine C with and IC_{50} (LoVo) of 30ng/mL.

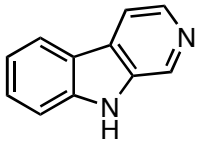
Fig. (**15**). Indole alkaloids isolated from cyanobacteria.



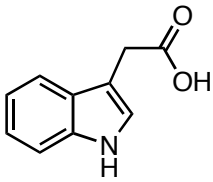
R = H: Bauerine A
R = Cl: Bauerine B



Bauerine C



Norharmane

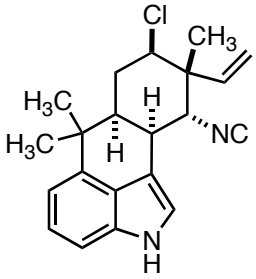


Auxin

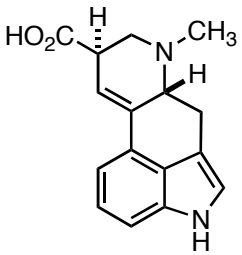
In 2005, a report by Volk of the Universität Kiel, Germany appeared describing the identification of norharmane of *Nodularia harveyana*.[126] This is the

first identification of this compound from cyanobacteria. Norharmane is known in higher plants, bacteria and even in humans. Volk reported anticyanobacterial activity[127] of this compound against both filamentous and unicellular cyanobacteria, whereas no activity against green algae could be determined. [128,129] This is in agreement with earlier reports on the isolation of harmane from a marine *Pseudomonas* species by Murakami and coworkers, who also showed anticyanobacterial activity with green algae being unaffected.[130] These authors also suggested the use of harmane for the control of toxic algal blooms.[130] Given the known co-mutagenicity of harmane,[131,132] this suggested approach for algal bloom control could be questioned. The import plant hormone (phytohormane) auxin or indolo acetic acid was also recently identified in cyanobacteria of the genus *Nostoc*.[133] The production and release of such phytohormones by cyanobacteria living in symbiotic or parasitic environments could possess significant ecological consequences.

Fig. (**16**). Hapalindole A, isolated from *Hapalosiphon fontinalis* is structurally related to lysergic acid.



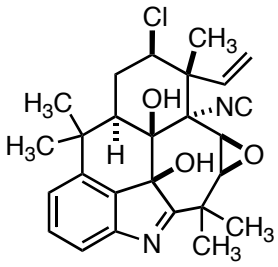
Hapalindole A



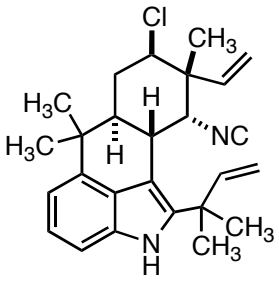
Lysergic acid

A class with structurally most remarkable indole alkaloids is based on hapalindoles and fischerindoles. Hapalindole A was isolated in 1984 from the blue green algae, *Hapalosiphon fontinalis* Fig. (**16**).[134] Interestingly, the isolation yield was rather high (0.58 % of dry cell weight). NMR spectroscopy complemented with UV and IR techniques were used to elucidate the fascinating structure of this metabolite containing a tetracyclic structure with isonitrile and chlorine substituents.[134] Hapalindole was reported to display antialgal and antimycotic properties, which point to the ecological role of this extracellular substance. In a subsequent publication, Moore and coworkers together with researchers from Eli Lilly reported the characterization of eighteen new hapalindole derivatives from the same organism.[135] All structures show the indole ring connected to a monoterpene unit, mainly with structural variation on the cyclohexane ring. Interestingly, the structure of hapalindole shares similarities to lysergic acid, a precursor to the ergot alkaloids.[134,135]

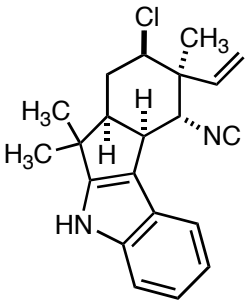
Fig. (**17**). The ambiguines and fischerindoles, members of the hapalindole family of indoles isolated from cyanobacteria.



Ambiguine Isonitrile D



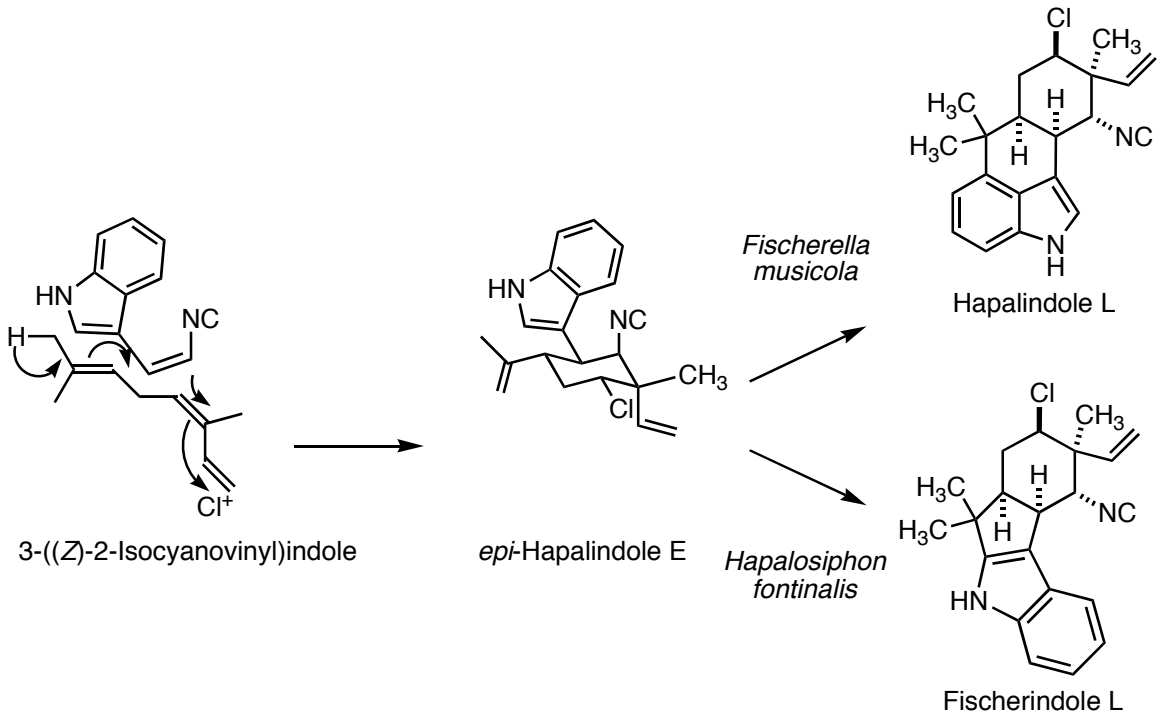
Ambiguine Isonitrile A



Fischerindole L

Ambiguine isonitrile can be considered a more complex derivative of hapalindole, containing a densely functionalized, hexacyclic framework Fig. (**17**).[136] In fact, prenylation of the C(2) of hapalindole followed by ring closure and oxidation would result in ambiguine isonitrile D. This hypothesis is supported by the isolation of putative intermediates such as ambiguine isonitrile A. These complex indole metabolites display fungicidal activities against several fungi in a soft-agar disc-diffusion assay.[136,137] Fischerindole L, isolated from the terrestrial blue-green alga *Fischerella musciola*, can be considered isomeric to hapalindole A, but possesses a hexahydroindeno[2,1-*b*]indole and isonitrile and chlorine functionalities. The isolation of fischerindole L could provide insights into the biosynthesis of these cyanobacterial indole alkaloids.[138] In fact, Moore and coworkers suggest that an isonitrile derived from tryptophan is fused to a geranyl pyrophosphate derivative via a chloronium ion-induced condensation Fig. (**18**).[138]

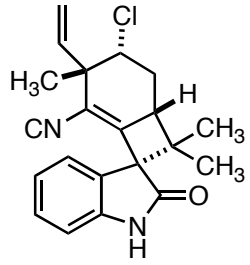
Fig. (**18**). Hypothetic biogenesis of hapalindole and fischerindole L from 3-((Z)-2-isocyanovinyl)indole as suggested by Moore and coworkers.[138,139]



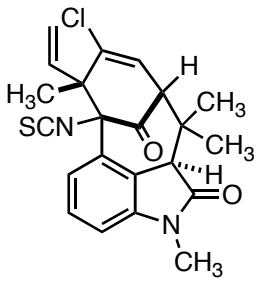
This biosynthetic proposal is fostered by the isolation of a hapalindole isomer (*epi*-hapalindole E) by Schwartz and coworkers from *Fischerella* ATCC 53558.[50] This structure was initially misassigned and later corrected by Moore and coworkers.[139] This compound could then be transformed into both fischerindole L and hapalindole L by electrophilic aromatic substitution. Interestingly, the formation of *epi*-hapalindole E can be rationalized by 3-((*Z*)-2-isocyanovinyl)indole as starting material. It is interesting to point out that this isomer was isolated from a *Pseudomonas* species.[140] It remains unknown to date, whether this compound is

the biogenetic precursor to the hapalindole and fischerindole families of natural products.

Fig. (**19**). The welwitindolines, structurally interesting members of the hapalindole series of natural products..[139]



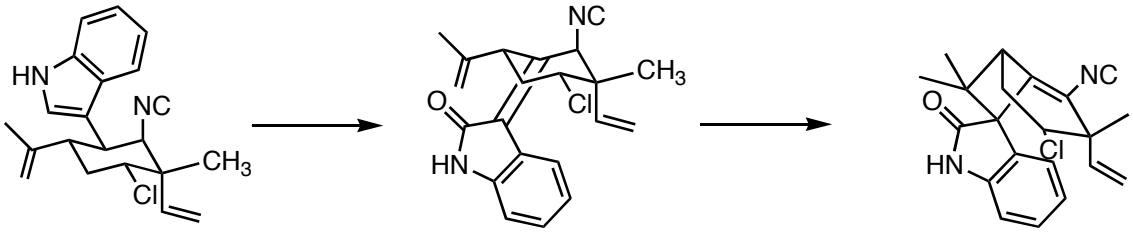
Welwitindoline A isonitrile



N-Methylwelwitindolinone C isothiocyanate

Another derivative of the hapalindole series are the welwitindolinones, isolated from *Hapalosiphon weilwitschi* Fig. (**19**).[139] These compounds are presumed as responsible for some of the interesting biological properties of these strains of blue-green algae such as multiple-drug-resistance reversing activity combined with insecticidal properties. Clearly, welwitindoline A isonitrile is derived from the parent structure but contains a unique cyclobutane oxindole core. Several derivatives were isolated including rearranged ketones such as *N*-methylwitindolinone C isothiocyanate.[139]

Fig. (**20**)**.** Biogenetic hypothesis for the formation of welwitindolinone A isonitrile from the isolated intermediate, epi-hapalindole E.[139]



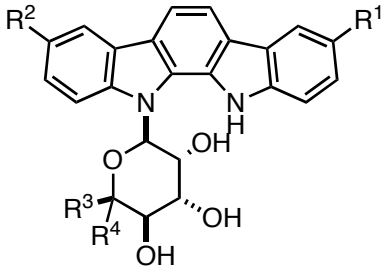
epi-Hapalindole E

welwitindolinone A isonitrile

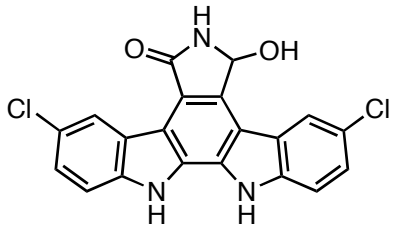
The biosynthesis of welwitindoline can be explained by a common intermediate, *epi*-hapalindole E, derived from 3-((*Z*)-2-isocyanovinyl)indole as discussed above Fig. (**20**). Oxidation and tautomerization would furnish an indoline intermediate, which could undergo the formation of the cyclobutane oxindole core welwitindoline. The exact molecular mechanism of the biogenesis, however, remains to be elucidated.

Another class of important indole derived metabolites are the tjipanazoles Fig. (**21**), isolated from *Tolypothrix tjipanasensis*.[141] These compounds share the same indolo[2,3-a]carbazole framework as other bioactive compounds such as staurosporine,[142-144] rebeccamycin (antitumor)[145,146] or the synthetic enzastaurin (LY317615, anti diabetes).[147,148] Unlike these derivatives from actinomycetes or slime molds, the tjipanazoles generally lack the pyrrolo[3,4-c] ring. The indolo[2,3-a]carbazole scaffold of the tjipanazoles is chlorinated to a varying degree and is glycosylated with 6-deoxy-D-gulose (Tjipanazole A1, C1, C2 and G1) and L-

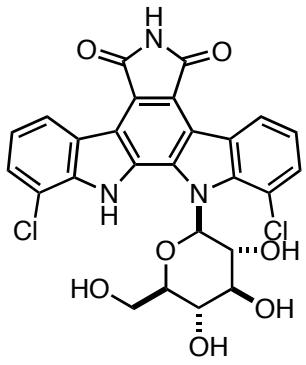
rhamnose (tjipanazoles A2, C3, C4 and G2). The biological activity of these compounds is characterized by antifungal properties, especially against phytopathogenic fungi such as rice blast and leaf rust wheat infections. The tjipanazole show only very weak activity against leukemia and solid tumor cell lines and no protein kinase C inhibition up to 10^{-6} M concentration. This is in sharp contrast to staurosporin/rebeccamycin, which is both an antitumor agent and a protein kinase C inhibitor.[143,145,146] The difference in biological activity is explained by the pyrrolo[3,4-c] ring of rebeccamycin and analogs, which is essential for their powerful biological properties.



Tjipanazole	R^1	R^2	R^3	R^4
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



Tjipanazole J



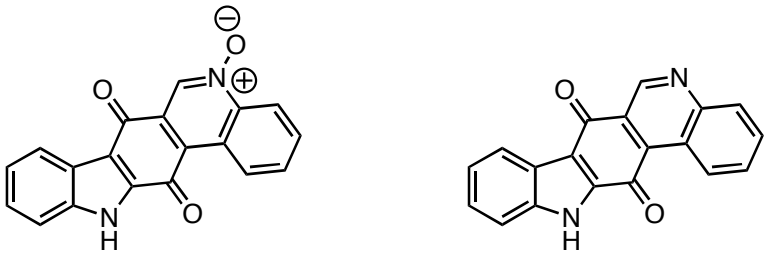
Rebeccamycin

Fig. (**21**). Tjipanazole, metabolites from *Tolypothrix tjipanasensis*.

In the context of a screening program of cyanobacterial metabolites against *Plasmodium falciparum*, the most virulent strain of plasmodia to humans, two new

indoloquinones, calothrixin A and B, were isolated and their structure determined Fig. (**22**).[149] 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 (58 nM vs. 180 nM), but still retained sub micromolar activity. The well-known antimalarial agent chloroquine displayed an IC_{50} value of 83 nM. These indoloquinoline metabolites also inhibit the growth of HeLa cancer cell lines, with an IC_{50} of 40 nM was measured for calothrixin A.

Fig. (**22**)**.** Calothrixin A and B, antitumor antimalarial quinones from *Calothrix* sp.

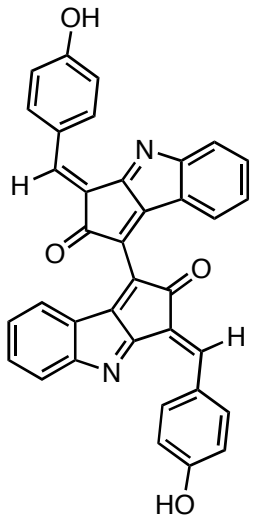


Calothrixin A Calothrixin B

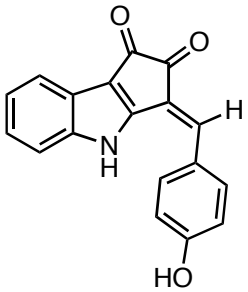
Cyanobacteria populate many different habitats. In particular, they evolved either to cope with little sunlight (i.e. *Planktothrix rubescens* lives 10-12 m below water surface) to being exposed to bright sunlight. In particular the latter case requires epilithic cyanobacteria to deal with an overexposure to UV radiation.[150,151] For example, on so-called *inselbergs*, isolated mountains surrounded by the sea, a unique vegetation occurs.[152] As there are no higher plants found for example on the Mitaraka inselberg in French Guyana, epilithic cyanobacteria, forming thick mats in

polysaccharide matrices, are supposed to be the only living organisms under intense solar radiation.[152] In order to meet this challenge, such cyanobacteria produce polyconjugated indole pigments as suncreens. One of these pigments, scytonemin, was reported over 100 years ago, but the structure was only characterized in 1993 Fig. (**23**).[153] It contains a dimeric structure probably derived of tryptophan and polyketide biosynthetic pathways. Later, a postulated intermediate, nostodione was isolated from a *Nostoc* species.[154] Nostodione, which was also obtained in a different study by ozonolysis of scytonemin,[153] could be reductively dimerized to the dimeric parent structure, scytonemin. Another indole, prenostodione, was isolated and postulated to serve as precursor to nostodione.[155] However, another possibility of its formation could also be oxidation of the dione fragment of nostodione in the course of the isolation procedure.

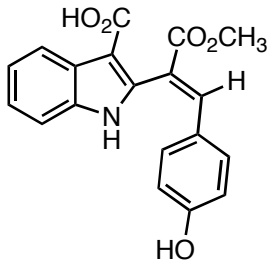
Fig. (**23**)**.** Cyanobacterial pigments from *Scytonema* sp (scytonemin[153]), *Nostoc commune* (nostodione[154]) and *Nostoc* sp (prenostodione[155]).



Scytonemin



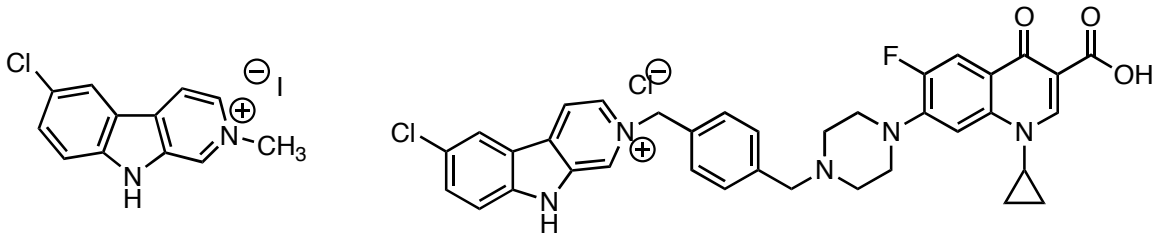
Nostodione



Prenostodione

The carbolinium alkaloid nostocarboline was isolated from the freshwater cyanobacterium *Nostoc* 78-12A, its structure characterized and synthesized Fig. (**24**). [156] This quaternary compound contains a chlorine atom, which is unusual for a freshwater metabolite. Nostocarboline is a potent cholinesterase inhibitor, which is an enzyme targeted in the treatment of Alzheimer's disease. In a subsequent publication, nostocarboline was shown to be an efficient algicide, inducing rapid killing of both prokaryotic and eukaryotic photosynthetic organisms. [157] However, nostocarboline was not active against normal non-photosynthetic pathogenic bacteria and fungi pointing to a potential inhibition in photosynthesis. In order to broaden the activity, the natural product hybrid[110], between the known antibacterial agent ciprofloxacin and nostocarboline was prepared. This compound shows a broad and unprecedented spectrum of activity thus combining the biological activity of both fragments.

Fig. (**24**). Nostocarboline, a natural carbolinium compound, and a synthetic natural product hybrid.



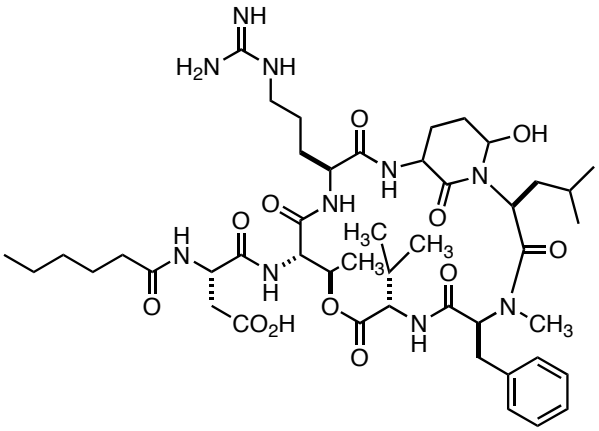
Nostocarboline

Nostocarboline/ciprofloxacin hybrid

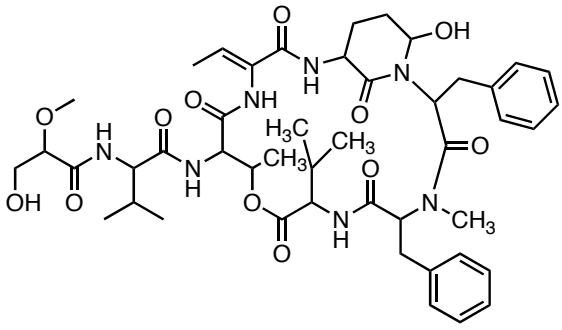
5. Protease inhibitors

Another important class of cyanobacterial metabolites consists of protease inhibitors Fig. (**25**).[158,159] These depsipeptides are characterized by an aminohydroxypiperidone (Ahp) unit, a *N*-methylated amino acid, a threonine, where the lactone ring is formed, and an attached peptidic side-chain, which is often terminated by either hydrophobic fatty acids or polar acids. Up to now, many of these depsipeptides have been isolated from cyanobacteria. One of the first members described in the literature are the cyanopeptolins A-D[160] featuring basic amino acid (Arg in Cyanopeptolin A, Lys in Cyanopeptolin B, *N*-Me-Lys in Cyanopeptolin, *N,N*-Dime-Lys in Cyanopeptolin), Ahp, *N*-Me-Phe as well as a terminal hexanoic acid.

Fig. (**25**)**.** Cyanopeptolin A isolated from *Microcystis* PCC 7806.[160]



Cyanopeptolin A

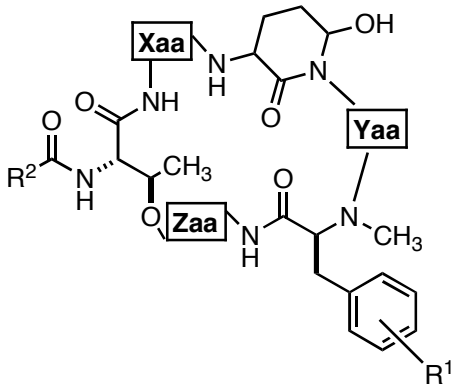


Dolastatin 13

These compounds have a striking structural similarity to dolastatin 13, isolated from the Indian Ocean shell-less mollusc *Dolabella auricularia*.[161] These

seemingly defenseless animals are only attacked by certain carnivorous predators, which is attributed to a very powerful chemical defense system of the sea slugs. These secondary metabolites are now thought to originate from the dietary sources of *Dolabella* such as cyanobacteria. Further support to this hypothesis is given by the structural similarity of dolastatin 13 to cyanopeptolins.[162]

Table (**1**). Structure/activity relationships for cyanobacterial depsipeptides against chymotrypsin and trypsin.



Depsipeptide	Ref.	Xaa	Yaa	Zaa	Chymotrypsin IC_{50} (μM)	Trypsin IC_{50} (μM)
Micropeptin 88-N	[163]	Tyr	Val	Ile	15	ni
Micropeptin SF909	[164]	Gln	Leu	Ile	5.0	ni
Micropeptin SD1002	[165]	Trp	Ile	Val	3.2	ni
Micropeptin T-1	[166]	Tyr	Phe	Val	3.0	ni
Micropeptin SD979	[165]	Tyr	Ile	Val	2.5	ni
Micropeptin 88-Y	[163]	Tyr	Val	Ile	1.3	ni
Cyanopeptolin 963A	[167]	Tyr	Leu	Val	0.9	ni
Planktopeptolin BL1125	[168]	Leu	Thr	Ile	0.8	ni
Nostopeptin BN920	[169]	Leu	Phe	Val	0.11	ni
Cyanopeptolin 954	[170]	Leu	Phe	Val	0.0044	ni
Micropeptin T-20	[171]	Tyr	Phe	Ile	0.0025	ni
Micropeptin SD944	[165]	Lys	Ille	Val	ni	8.5
Micropeptin SD999	[165]	Arg	Ile	Val	ni	4
Micropeptin 90	[172]	Arg	Phe	Val	ni	2.5
Cyanopeptolin S	[173]	Arg	Ile	Ille	ni	0.2
Micropeptin T-2	[166]	Lys	Phe	Val	ni	0.1
Micropeptin A	[174]	Lys	Leu	Val	ni	0.07
Oscillapeptin F	[175]	Hca	Ile	Ile	ni	0.2
A90720A	[176]	Arg	Leu	Val	ni	0.01

As over twenty of these depsipeptides were isolated from cyanobacterial sources, structure/activity relationships on their inhibitory action can be performed. In table (**1**), a selection of these compounds is given, with the variable amino acids, Xaa, Yaa and Zaa highlighted. Several requirements for activity become evident:

a. The key residue for protease inhibition consists of the aminohydroxypiperidone residue. This amino acid is conserved for all these depsipeptides.

b. All active compounds share a 19-membered lactone ring, with the amino acid Zaa acylates the β-OH group of Thr

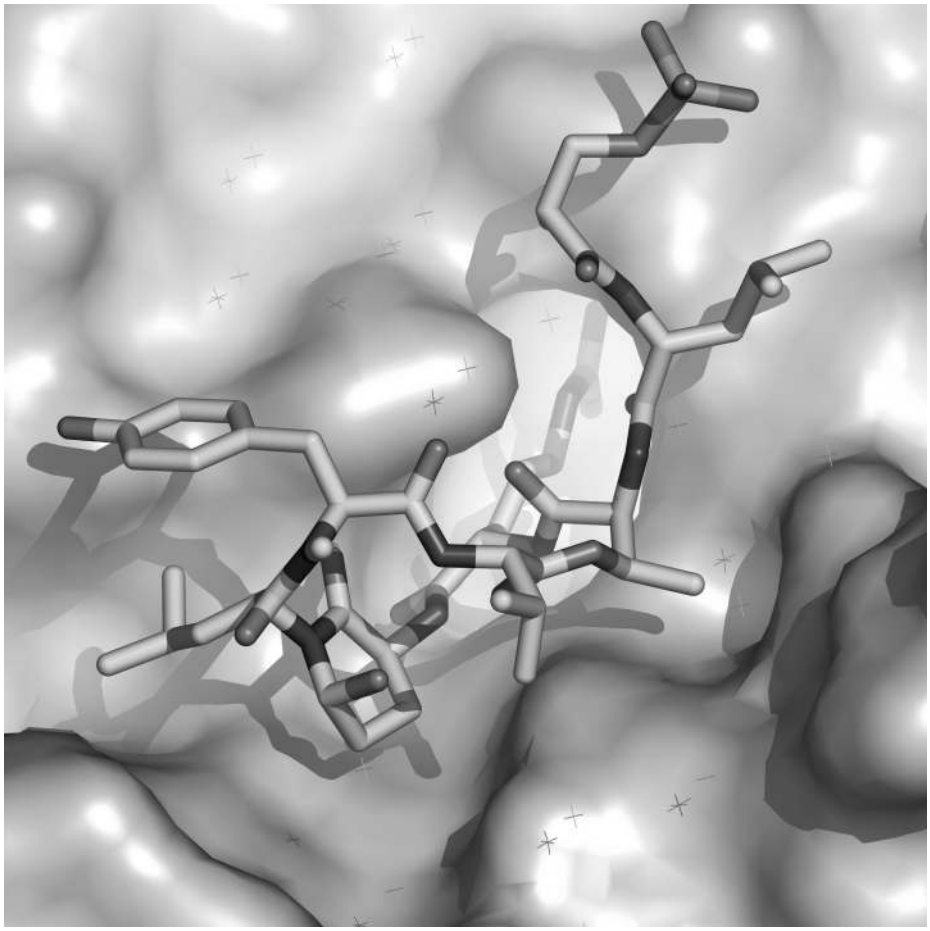
c. The selectivity for chymotrypsin is dictated by a hydrophobic amino acid adjacent to Ahp, mostly consisting of Tyr or Leu, with Gln and Trp only found once. The selectivity for trypsin is achieved by basic amino acids such as Arg or Lys. An exception is apparently oscillapeptin F with the highly unusual Hca amino acid.

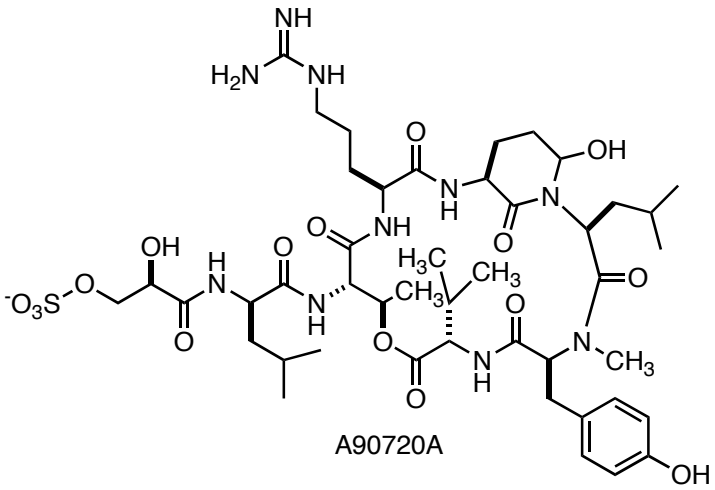
d. The range of activities is rather broad, spanning three orders of magnitude against chymotrypsin and four orders of magnitude for trypsin. The most potent derivatives are micropeptin T-20 (IC 50 = 2.5 nM against chymotrypsin) and A90720A (IC50 = 10 nM against trypsin).

The mode of action of the potent trypsin inhibitor A90720A[176] can be understood by inspection of its crystal structure complex with bovine trypsin Fig. (**26**).[177] Several interesting features can be observed. The arginine side chains

occupies trypsin's deep and narrow specificity pocket, and the terminal guanidinium group tightly binds to Asp189 and Ser 190 at the end of the pocket. If A90720A were a trypsin substrate, the arginine carbonyl group would be attacked by the Ser OH group of the catalytic triad. Whereas the CO group of the Arg of A90720A points in the oxyanion hole, the Ser-His part of the catalytic triad are 2.77 Å away from the CO of the inhibitor. This distance combined with the strong and defined binding of the Ahp residue within the inhibitor leading to a well-defined cyclic conformation, inhibits the enzymatic action. Additional binding energy is delivered by the hydrophobic region of A90720A involving *N*-Me-Tyr and a Leucine residue. The Ahp residue is essential for the inhibitory action by the transannular hydrogen bonds to the Val residue, which determined the binding conformation and thus prevent dissociation. The authors of this structural biology work stated that replacement of the cyclic Leu residue by and aromatic group will lead to even greater inhibitory potency.

Fig. (**26**). Part of the X-Ray crystal structure of the A90720A/bovine trypsin complex (protein data bank: 1TPS).)[177]





A90720A

6. Conclusion

This review gave an overview of the large variety of different cyanobacterial metabolites featuring polyketide, alkaloid, peptides and terpene fragments and any combination thereof. Whereas some metabolites are of general concern due to their toxicity such as microcystins, saxitoxins or anatoxins, other display significant pharmaceutical potential as “drugs from the sea” like the cryptophycins. Some of these compounds might be produced for deterrence purposes or to render cyanobacteria unattractive as a food source for grazer (by strong protease inhibitors). Other compounds that act as iron chelators such as anachelin or as pigments (scytonemin) have other functions of vital interest to cyanobacteria populating habitats with extreme conditions such as bare rocks or the open ocean. All these metabolites demonstrate how small molecules can secure evolutionary advantage of the producing organisms (in this case cyanobateria) to survive in vastly different habitats. Moreover, these fascinating structures combined with powerful biological activities are inspiration to the chemist for the understanding of natural phenomena on a molecular level and for the development of molecular solution to the problems our society faces today.

7. References

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