Isolation and characterization of halogenated natural products from the cyanobacteria genera *Hapalosiphon* and *Fischerella*

Dissertation
zur Erlangung des
Doktorgrades der Naturwissenschaften (Dr. rer. nat.)

der

Naturwissenschaftlichen Fakultät I – Biowissenschaften –

der Martin-Luther-Universität
Halle-Wittenberg,

vorgelegt

von Herrn Tomasz Chilczuk
geb. am 01.09.1989 in Oppeln

Tag der mündlichen Qualifikation:	26.11.2021
Dekan:	Prof. Dr. Dietrich Nies
1. Berichterstatter:	Prof. Dr. Timo H. J. Niedermeyer
2. Berichterstatter:	Prof. Dr. Markus Nett
3. Berichterstatter:	Prof. Dr. Björn Junker

Zusammenfassung

Naturstoffe werden seit Jahrhunderten vom Menschen für die Behandlung verschiedener Krankheiten verwendet. Obwohl die Investitionen der Pharmaunternehmen in die Naturstoff-Forschung in den letzten Jahrzehnten zurückgegangen sind, spielen Naturstoffe nach wie vor eine wichtige Rolle in der Entwicklung neuer Arzneimittel. Eine Quelle für neue bioaktive Naturstoffe von zunehmender Bedeutung sind Cyanobakterien. Daher war es das Ziel der vorliegenden Arbeit, neue Naturstoffe aus Cyanobakterien für die Arzneimittelentwicklung zu identifizieren und zu charakterisieren, wobei drei verschiedene Ansätze verfolgt wurden.

(I) Ein strukturgeleitetes Screening mit der Software HaloSeeker wurde durchgeführt, die der Identifizierung neuer halogenierter Naturstoffe dient. Das Screening zeigte das Vorhandensein mehrerer unbekannter halogenierter Verbindungen im Extrakt des Cyanobakteriums *Fischerella ambigua* (Näg.) Gomont 108b an. Drei neue Naturstoffe, Tjipanazole K, L und M, sowie die beiden bereits bekannten Tjipanazole D und I wurden aus *F. ambigua* (Näg.) mittels Flash-Chromatographie und semi-präparativer HPLC isoliert. Die Strukturen der Verbindungen wurden mittels HRMS und NMR aufgeklärt. Wir konkretisierten den Tjipanazole-Biosyntheseweg aufgrund neuer Erkenntnisse über den biosynthetischen Gencluster sowie über die Strukturen der im Stamm nachgewiesenen Tjipanazole. Aufgrund der strukturellen Ähnlichkeiten aller Tjipanazole mit bereits bekannten Inhibitoren des Brustkrebs-Resistenz-Transporters ABCG2 wurden alle Verbindungen hinsichtlich ihrer Fähigkeit, ABCG2 zu hemmen, charakterisiert. Tjipanazole M zeigte eine ähnlich starke hemmende Wirkung gegen ABCG2 wie der bekannte Inhibitor Acyriaflavin A. Die durchgeführte Struktur-Wirkungsbeziehungsstudie zeigte, dass der Pyrrolo[3,4-c]-Ring eine entscheidende Voraussetzung für die ABCG2 hemmende Wirkung in dieser Substanzklasse ist.

(II) Eine Extraktsammlung von Cyanobakterien wurde mit Hilfe eines Screenings auf immunmodulierende Naturstoffe untersucht. Zwei neue Naturstoffe, Hapalindole A-Formamid und Hapalindole J-Formamid, wurden zusammen mit den drei zuvor beschriebenen Hapalindolen A, D und M aus dem Cyanobakterium *Hapalosiphon* sp. CBT1235 mittels semi-präparativer HPLC isoliert. Die Strukturen aller Hapalindole wurden mit Hilfe von HRMS und NMR aufgeklärt. Die Auswirkungen der Verbindungen auf die T-Zellproliferation und T-Zellapoptose wurden untersucht. Alle Hapalindole

unterdrückten die T-Zellproliferation, wobei Hapalindole A die stärkste hemmende Aktivität zeigte. Obwohl Hapalindole A apoptotische Aktivität gegen T-Zellen zeigte, war die Abschwächung der T-Zellproliferation bei bis zu 10-fach niedrigeren Konzentrationen messbar, was es zu einem potenziellen Kandidaten für die Entwicklung neuer immunmodulierender Medikamente macht.

(III) Ein Screening einer Extraktsammlung von Cyanobakterien wurde durchgeführt, um Inhibitoren des Quorum Sensing Systems von *Serratia marcescens* zu identifizieren. Es wurde kein Quorum Sensing hemmender Extrakt entdeckt. Jedoch wurde festgestellt, dass ein Extrakt von *Fischerella ambigua* (Näg.) Gomont 108b die Produktion des Quorum Sensing regulierten Pigments Prodigiosin in *S. marcescens* erhöht. Aufgrund dieser faszinierenden Aktivität, wurden die beiden neuen Naturstoffe, Ambigol D und E, sowie die beiden bereits bekannten Ambigole A und C mittels Flash-Chromatographie und semi-präparativer HPLC aus *F. ambigua* isoliert. Die Strukturen der Verbindungen wurden mittels HRMS und umfangreichen NMR-Experimenten aufgeklärt. Alle Ambigole erhöhten die Prodiginin-Produktion von *Serratia* sp. ATCC 39006, wobei Ambigol C die stärkste Aktivität zeigte. Darüber hinaus zeigten Ambigol A und D antibakterielle Aktivität gegen *Serratia* sp. ATCC 39006. Eine Genexpressionsanalyse des Prodigiosin-Biosynthese-Genclusters ergab, dass Ambigol C die Prodigiosin-Produktion in *Serratia* sp. ATCC 39006 nicht erhöht, indem es die Transkriptionsrate der Prodigiosin-Biosynthesegene verstärkt. Daher wurde der Einfluss von Ambigol C auf das Transkriptom von *Serratia* sp. ATCC 39006 mit Hilfe von RNA-Seq und anschließender differentieller Genexpressions- und funktioneller Anreicherungsanalyse untersucht.
Der Wirkmechanismus für die beobachtete Aktivität der Ambigole konnte anhand der Transkriptomanalyse nicht ermittelt werden. Unsere Ergebnisse deuten jedoch darauf hin, dass Ambigol C einen großen Einfluss auf die Translationsmaschinerie und die Präkursorversorgung der Prodigiosin-Biosynthese von *Serratia* sp. ATCC 39006 hat.

Alle drei Screening-Ansätze waren erfolgreich in der Identifizierung neuer bioaktiver Naturstoffe aus Cyanobakterien und veranschaulichen, dass Cyanobakterien in der Tat eine produktive Quelle für bioaktive Naturstoffe sind. Die mögliche Verwendung der isolierten Verbindungen als Kandidaten für die Arzneimittelentwicklung oder andere Anwendungsbereiche muss in zukünftigen Studien noch untersucht werden.

Summary

Natural products have been used by humans for the treatment of various disease since centuries. Although the investments of pharmaceutical companies in natural product research declined over the last decades, natural products still play a major role in the development of new drugs. An increasingly recognized source for new bioactive natural products are cyanobacteria. Thus, the present thesis aimed to identify and characterize new natural products from cyanobacteria for drug development purposes utilizing three distinct approaches.

(I) A chemistry-guided screening using the software HaloSeeker dedicated to the identification of new halogenated natural products was conducted. The screening indicated the presence of several unknown halogenated compounds in the extract of the cyanobacterium *Fischerella ambigua* (Näg.) Gomont 108b. Three new natural products, tjipanazole K, L, and M, as well as the two already known tjipanazoles D and I were isolated from *F. ambigua* (Näg.) using flash chromatography and semi-preparative HPLC. The structures of the compounds were elucidated using HRMS and NMR. We substantiated the putative tjipanazole biosynthetic pathway based on new insights into the biosynthetic gene cluster as well as the structures of the tjipanazoles detected in the strain. Due to the structural similarities of all tjipanazoles to already known inhibitors of the breast cancer resistance transporter ABCG2, all compounds were characterized for their capability to inhibit ABCG2. Tjipanazole M exhibited similar potent inhibitory activity against ABCG2 as the known inhibitor acyriaflavin A. The conducted structure-activity relationship study showed that the pyrrolo[3,4-c] ring is a critical requirement for the ABCG2 inhibitory activity in this compound class.

(II) A cyanobacterial extract collection was screened for immunomodulating natural products. Two new natural products, hapalindole A-formamide and hapalindole J-formamide, along with the three previously described hapalindoles A, D, and M were isolated from the cyanobacterium *Hapalosiphon* sp. CBT1235 using semi-preparative HPLC. The structures of all hapalindoles were elucidated by HRMS and NMR. The effects of the compounds on T cell proliferation and T cell apoptosis were investigated. All hapalindoles suppressed T cell proliferation, with hapalindole A showing the most potent inhibitory activity. Although hapalindole A showed apoptotic activity against T cells, the attenuation of T cell proliferation was measurable at up to 10-fold lower

concentrations, making it a potential candidate for the development of new immunomodulating drugs.

(III) A screening of a cyanobacteria extract collection was conducted to identify inhibitors of the quorum sensing system of *Serratia marcescens*. No quorum sensing inhibiting extract was detected. However, an extract of *Fischerella ambigua* (Näg.) Gomont 108b was found to increase production of the quorum sensing-regulated pigment prodigiosin in *S. marcescens*. Intrigued by this activity, the two new natural products, ambigol D and E, as well as the two already known ambigols A and C were isolated from *F. ambigua* using flash chromatography and semi-preparative HPLC. The structures of the compounds were elucidated using HRMS and extensive NMR experiments. All ambigols increased the prodiginine production of *Serratia* sp. ATCC 39006, with ambigol C showing the most potent activity. Moreover, Ambigol A and D showed antibacterial activity against *Serratia* sp. ATCC 39006. A gene expression analysis of the prodigiosin biosynthetic gene cluster revealed that ambigol C does not increase prodigiosin production in *Serratia* sp. ATCC 39006 by elevating the transcription rate of prodigiosin biosynthesis genes. Thus, the influence of ambigol C on the transcriptome of *Serratia* sp. ATCC 39006 was examined using RNA-Seq and subsequent differential gene expression and functional enrichment analyses. The mode-of-action for the observed activity of the ambigols could not be determined based on the transcriptome analysis. However, our results indicate that ambigol C has a major impact on the translation machinery and the precursor supply of prodigiosin biosynthesis of *Serratia* sp. ATCC 39006.

All three screening approaches were successful in identifying new bioactive natural products from cyanobacteria and illustrate that cyanobacteria are indeed a prolific source for bioactive natural products. The potential use of the isolated compounds as candidates for drug development or other applications remains to be investigated in future studies.

Table of contents

1 Introduction

1.1 Cyanobacteria as a prolific source for bioactive natural products

Chemical substances derived from living organisms, such as plants, bacteria, fungi and animals, are called natural products and have been used by humans for medical purposes since ancient times. They are used as a direct source for therapeutic agents in form of pure compounds or defined mixtures, a source for the development of semi-synthetic drugs, or prototypes for the design of lead molecules.[1] In the past 39 years 23.5 % of all approved therapeutic agents were natural products, natural product derivatives or botanical drugs. This percentage rises to 37.9 %, if synthetic drugs with a natural product pharmacophore and mimics of natural products that feature a natural product pharmacophore but have been discovered differently are being taken into account.[2]

Most recently, cyanobacteria gained attraction as a prolific source for bioactive natural products, since many cyanobacterial compounds with novel structural scaffolds and potent bioactivities have been discovered.[3–5] Cyanobacteria represent one of the most ancient life forms on earth with discovered fossils of cyanobacteria-like organisms dating back 3.5 billion years.[3] They colonize various and sometimes extreme environments around the world, resulting in a high morphologic, physiologic and metabolic diversity.[4] Their widespread appearance in different ecological niches and long evolutionary history could explain their production of chemical diverse natural products, such as alkaloids, depsipeptides, lipopeptides, macrolides, terpenoids, polysaccharides, and lipids (**Table 1**).[5] Around half of the known cyanobacteria metabolites are peptides or peptide derivatives that are produced by nonribosomale petide synthetases (NRPS) or polyketide synthases (PKS), or a combination of both systems.[6,7] Notably, 70 % of all studied genome sequenced cyanobacterial strains exhibit NRPS and PKS gene clusters in their genomes.[8] However, only 20 % of all known cyanobacterial compound families can be linked to specific identified biosynthetic gene clusters, showing that the vast majority of biosynthetic pathways of cyanobacterial metabolites is still unknown.[9] The discovery of many derivatives of one parent compound is not uncommon in cyanobacteria. Over 270 different microcystins,[10] 82 cyanopeptolins,[6] and over 30 hapalindoles[11] have been isolated from different cyanobacteria, showing a high natural diversity within a given compound family.

Table 1. Examples for cyanobacterial compounds according to their respective chemical classes and producer strains.

Chemical class	Compounds (examples)	Producing cyanobacterium
Alkaloids	hapalindoles, ambiguine isonitrils	*Hapalosiphon fontinalis*,[12] *H. hibernicus*[13]
Depsipeptides	cryptophycins, cyanopeptolins	*Nostoc* sp.,[14,15] *Microcystis* sp.[16]
Lipopeptides	anabaenolysins, majusculamides	*Anabaena* sp.,[17] *Lyngbya majuscula*[18]
Macrolides	scytophycins, biselyngbyasides	*Scytonema pseudohofmanni*,[19] *Lyngbya* sp.[20]
Terpenoids	comnostins, norabietanes	*Nostoc commune*,[21] *Microcoleous lacustris*[22]
Polysaccharides	calcium spirulan, cyclodextrins	*Arthrospira platensis*,[23] *Tolypothrix byssoidea*[24]
Lipids	lyngbic acid, lyngbyoic acid	*Lyngbya majuscula*[25,26]

Cyanobacteria are famous for the formation of blooms in freshwater bodies all over the world and their production of toxins, which pose a threat to the health of humans and animals.[27] Apart from toxins, it has become more and more clear that cyanobacteria have the ability to produce natural products with antibacterial, antifungal, antiviral, antiprotozoal, molluscicidal, anticancer, immunosuppressive, anti-inflammatory, antioxidant activities, as well as the ability to inhibit proteases, and histone deacetylases.[9,28,29]

Some cyanobacterial natural products with promising bioactivities are or were in clinical trials (**Figure 1**). The most successful cyanobacterial natural products with a medical application are the peptidic dolastatins. They were isolated from the genera *Lyngbya* and *Symploca* and show antiprotozoal and antiproliferative activities.[30–32] Both activities are caused by binding to tubulin, preventing the microtubule assembly during mitosis.[32,33] Especially dolastatin 10 (**1**) showed promising *in vitro* activities as a potential anticancer drug. Thus, phase I and II clinical trials were conducted with this compound as a single therapeutic agent, but a phase III clinical trial was dropped due to toxic effects.[34] Moreover, the dolastatin 10 synthetic analogue soblidotin (**2**) showed promising results as an effective drug against MX-1 breast carcinoma and LX-1 lung carcinoma in p53 normal and mutant cells.[35,36] Soblidotin (**2**) reached phase II clinical trials, but did not enter a phase III clinical trial due to a lack of efficacy during trials.[37]

The most successful cyanobacterial natural product is a synthetic analogue of **1**, monomethyl auristatin E (**3**). This compound passed all clinical trials as an antibody drug conjugate for the treatment of Hodgkin's lymphoma or systemic anaplastic large cell

lymphoma,[38] making it the first approved drug (Brentuximab vedotin) based on a cyanobacterial natural product. Since then, more than 30 antibody drug conjugates with monomethyl auristatin E (**3**) or monomethyl auristatin F (**4**) as a drug, were evaluated in clinical trials.[37] Two analogues of the peptide dolastatin 15, cemadotin (**5**) and synthadotin/tasidotin (**6**), were also examined as potential anticancer drugs in clinical studies. However, follow up clinical trials on **5** were discontinued, whereas **6** has completed three phase II clinical trials.[39]

Further prominent molecules from cyanobacteria that are or were in clinical trials as potential therapeutic drugs are the cryptophycins. They are depsipetides with very high cytotoxicity based on their ability to inhibit microtubule assembly and were isolated from the cyanobacterium *Nostoc*.[14,15] The semi-synthetic analogue cryptophycin 52 (**7**) showed promising cytotoxic activities against multi-drug resistant tumor cells with adequate stability in aqueous solution compared to other cryptophycins.[40] Thus, **7** was chosen to undergo clinical evaluation. Cryptophycin 52 (**7**) reached phase II of clinical trials. However, due to a lack of efficacy *in vivo* and toxicity effects further investigations have been discontinued.[41] Although **7** failed in phase II clinical trials as a single therapeutic agent, there is still an interesting perspective for the usage of cryptophycins as antibody drug conjugates for the treatment of cancer.[42]

Moreover, neosaxitoxin (**8**), a derivative of saxitoxin, is a cyanobacterial natural product that was tested as a long-acting local anesthetic in phase I clinical trials.[43,44] Neosaxitoxin (**8**) is a highly potent neurotoxin that was isolated from various species from the cyanobacteria genera *Aphanizomenon*.[45–47] Studies showed that **8** as well as other saxitoxins function as reversible inhibitors of voltage-gated sodium channels, thereby blocking impulse generation and propagation in animals and humans.[48,49] Furthermore, studies revealed that the local anesthetic activity of **8** can be potentiated and prolonged, if it is administered in combination with other local anesthetics (e.g. bupivacaine) or vasoconstrictors (e.g. epinephrine).[43,50] In addition to the local anesthetic activity of **8**, Montero et al. showed that **8** inhibits the expression of inflammatory cytokines derived from macrophages, making **8** a potential candidate for the development of anti-inflammatory drugs.[51]

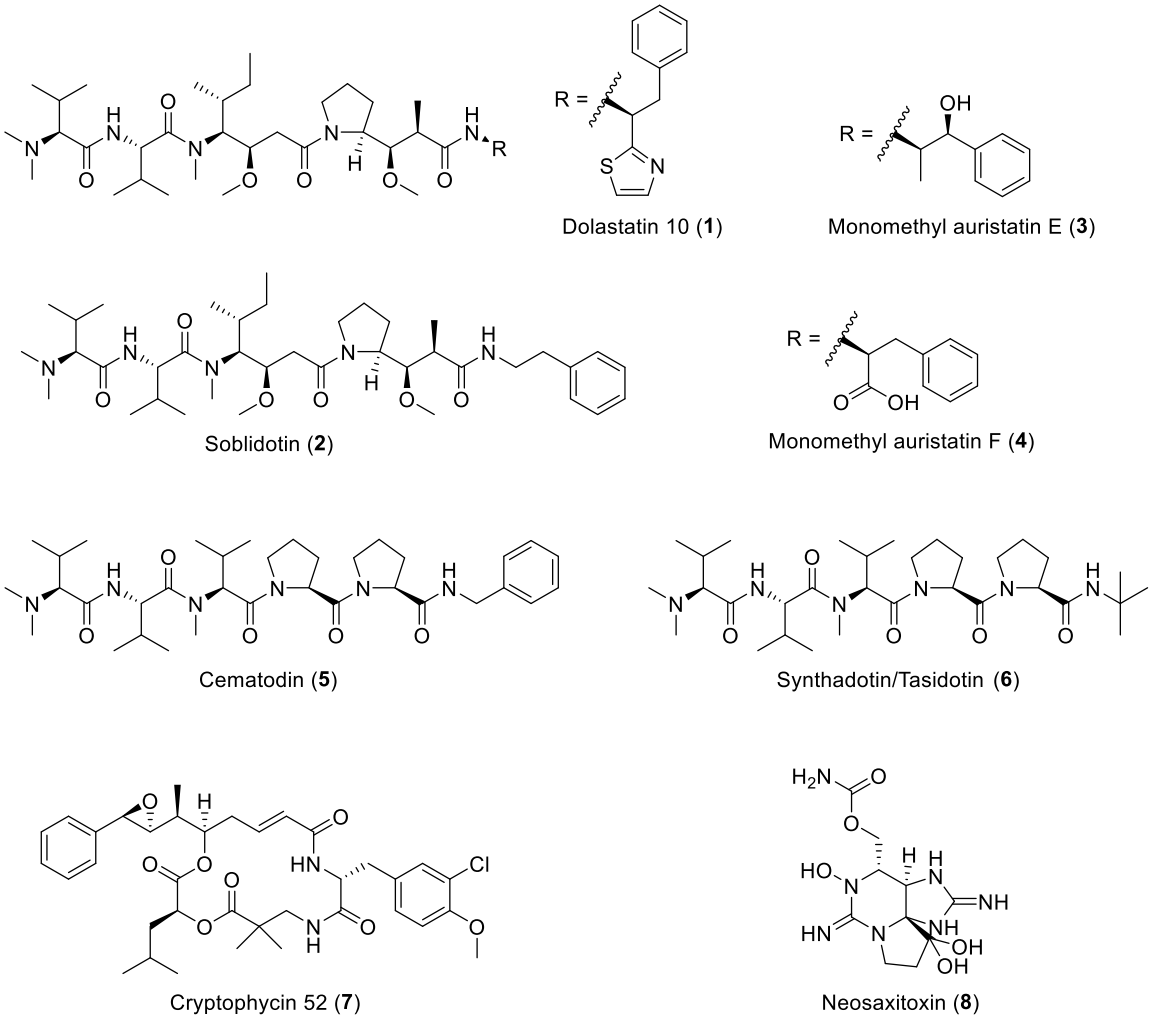


Figure 1. Selected cyanobacterial metabolites that are or were in clinical trials.

1.2 Halogenated natural products from cyanobacteria

Many halogenated natural products (HNPs) are biosynthesized by marine organisms, such as marine bacteria,[52,53] algae,[52,54–59] and marine invertebrates.[60–66] However, they are also produced by terrestrial plants,[67–71] fungi,[72–77] soil bacteria,[78–82] insects,[83–85] and higher animals.[86,87] Cyanobacteria synthesize a wide range of HNPs, including various peptides, indole alkaloids, and other chemical classes (**Table 2**).

HNPs exhibit various bioactivities including antibacterial, antifungal, antiparasitic, antiviral, anti-inflammatory, antitumor and molluscicidal activities.[88] The bioactivity of natural products is often depended on the presence of halogen substituents. For instance, the non-chlorinated derivative of cryptophycin 1, exhibited an 8-fold lower inhibitory activity against human cervical carcinoma cells than cryptophycin 1.[89]

Chlorodysinosin A is another cyanobacterial HNP from the aeruginosin family that showed increased enzyme inhibitory activities against thrombin, FVIIa and trypsin compared to its non-chlorinated variant.[90]

Table 2. Examples for halogenated compounds from cyanobacteria according to their respective chemical classes and producer strains.

Chemical class	Compounds	Producing cyanobacterium
Peptides	aeruginosins, cyanopeptolins, cryptophycins, microginins, lyngbyabellins	*Microcystis aeruginosa*,[91,92] *Scytonema hofmanni*,[93] *Nostoc* sp., [14] *Lyngbya majuscula*[94]
Indole alkaloids	ambiguines, hapalindoles, welwitindolinones, fischerindoles	*Hapalosiphon hibernicus*,[13] *H. fontinalis*,[12] *H. welwitschii*,[95] *Fischerella muscicola*[96]
Polychlorinated polyphenols	ambigols	*Fischerella ambigua*[97]
Glycolipids	bartolosides	*Nodosilinea* sp.[98]
Paracyclophanes	carbamidocyclophanes	*Nostoc* sp. [99]
Fatty acid amides	malyngamides	*Lyngbya majuscula*[100]
Indolocarbazoles	tjipanazoles	*Tolypothrix tjipanasensis*[101]

Chlorine is the halogen most frequently found in HNPs, followed by bromine, whereas fluorination and iodination are quite unusual in nature.[102] Enzymatic halogenation of natural products is often facilitated by hydrogen peroxide-dependent haloperoxidases or oxygen-dependent halogenases,[103] and corresponding enzymes have been found in cyanobacteria.[104,105] Halogenation of metabolites contributes to the high chemical diversity in cyanobacteria (**Figure 2**). Various HNP from cyanobacteria with one (**9**, hapalindole A), two (**10**, tjipanazole A1) or even six halogens (**11**, ambigol A) incorporated in their structure have been discovered. Further unusual modifications are the addition of several halogens to one carbon atom like in hectochlorin (**12**) and barbamide (**13**) or the incorporation of chlorine and bromine such as in the case of jamaicamide A (**14**).

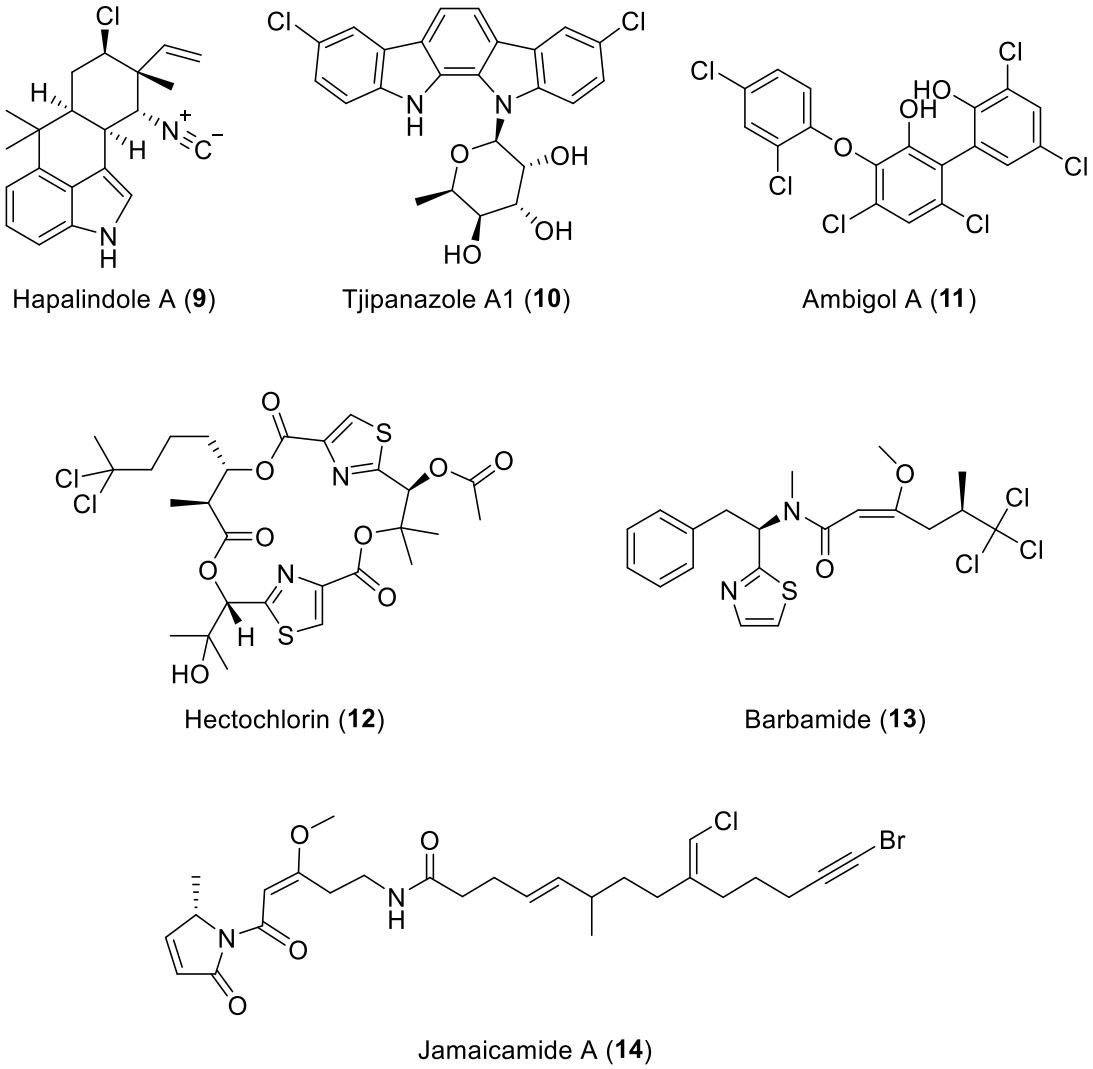


Figure 2. Selected halogenated natural products from cyanobacteria.

1.3 Immunomodulatory natural products from cyanobacteria

Immunity of multicellular organisms is a biological defense system against pathogenic agents, e.g. bacteria, fungi, parasites, viruses, cancer cells and toxins. The immune system can be classified into two categories, the non-specific and the specific immune system.[106] The non-specific immune system, also called innate immune system, is the first immunological mechanism for fighting against pathogenic agents that functions immediately or within hours. It is not able to recognize or "memorize" a specific pathogen if the organism is exposed to it another time.[107] Various defensive mechanism are part of the innate immune system. Anatomic barriers such as skin and mucous membranes or physiologic barriers like inhospitable pH, high body temperatures (fever), or the production

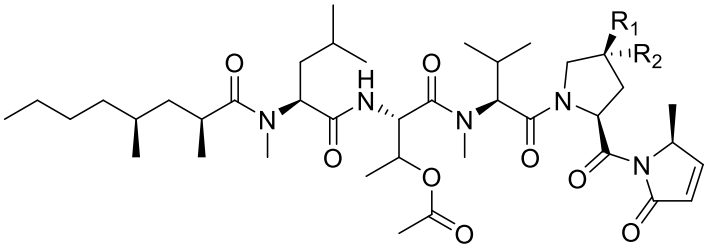
of antimicrobial molecules prevent the entry of pathogens into the host as well as their spreading within the body. Moreover, phagocytosis of pathogenic agents by macrophages, neutrophils, dendritic cells, mast cells, basophils, eosinophils or natural killer cells enables their removal. Many of the innate immunity cells also produce cytokines and present antigens to T and B cell in order to activate the specific immune system.[107]

The specific immune system is also referred to as the adaptive immune system and takes more time to develop than the innate immune system. The purpose of the adaptive immune system is the recognition and destruction of specific pathogens, as well as their quick elimination in subsequent infections. The adaptive immune response is mediated by T and B cells. T cells can recognize antigen fragments bound to the surface of antigen-presenting cells.[108] The recognition of antigen fragments stimulates T cells to differentiate into cytotoxic T cells and T helper cells, enabling the destruction of cells infected with pathogenic agents and the recruitment and activation of B cells, respectively. In contrast to T cells, B cells can be activated by soluble antigens and differentiate into antibody-secreting plasma cells or memory cells. While plasma cells are short-lived, memory cells can live for months, years or a lifetime.[108]

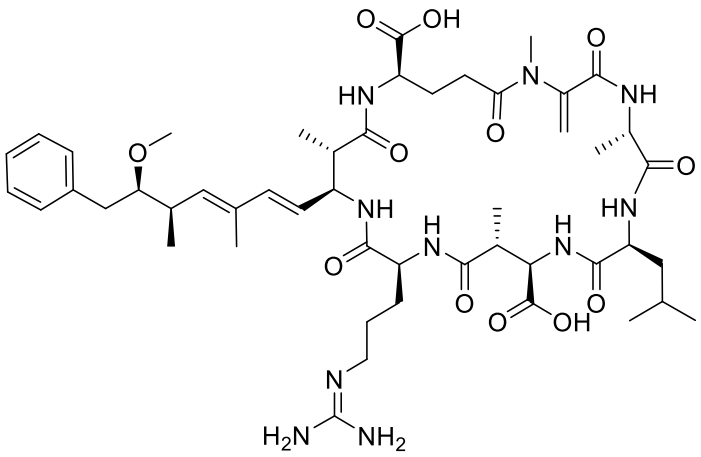
Immunomodulators are synthetic or natural substances that help to regulate or normalize the immune response by acting on different components of the immune system. Potential targets of immunomodulators are components from the innate immune system (e.g. macrophages, dendritic cells, natural killer cells, neutrophils) as well as from the adaptive immune system (e.g. T and B cells).[109,110] Immunomodulators can be further classified into immunostimulants and immunosuppressants. Immunostimulants enhance or activate components of the immune system and can be used against cancer and infectious diseases.[109,111] In contrast, immunosuppressants inhibit the immune system and are prescribed to treat autoimmune diseases or prevent the rejection of tissue and organ transplants.[112]

Various biomass extracts, commercial products as well as biomolecules from cyanobacteria are known to exhibit immunomodulatory activities. Investigations for immunomodulating activities of cyanobacterial extracts showed that extracts of *Arthrospira* exhibit immunostimulating activities,[113] whereas extracts of *Oscillatoria* and *Synechocystis* displayed immunosuppressive properties.[114] The protein C-phycocyanin from *Arthrospira* showed potent immunomodulating activities by activating macrophages, T and B cells,

stimulating cytokine production or inhibiting antibody and histamine production.[115] Further immunomodulating properties have been credited to the natural products microcolin A (**15**), microcolin B (**16**), and microcystin-LR (**17**) (**Figure 3**). Microcolin A (**15**) and microcolin B (**16**) are lipopeptides isolated from the cyanobacterium *Lyngbya majuscula* and were found to suppress T cell proliferation.[116] Microcystins are heptapeptides produced by various cyanobacteria including *Microcystis*, *Anabaena*, *Nostoc*, and *Planktothrix* species.[117] The most commonly found microcystin in cyanobacterial blooms is **17**.[118,119] Microcystin-LR (**17**) has been found to activate macrophages,[120] whereas other microcystin variants showed no immunomodulatory activity.[121] Although **17** exhibited immunomodulating properties, it is also known for its heptatoxic,[122] neurotoxic,[123] and genotoxic activities.[124]



Microcolin A (R_1 = OH, R_2 = H; **15**)
Microcolin B (R_1 = H, R_2 = H; **16**)



Microcystin-LR (**17**)

Figure 3. Selected natural products from cyanobacteria with immunomodulating activity.

1.4 An alternative strategy to fight drug-resistant bacteria

The discovery of penicillin in 1928 by Alexander Fleming and its use in the 1940s enabled the successful treatment of infections caused by Gram-positive bacteria.[125] Later, numerous antimicrobial agents such as cephalosporin, erythromycin and vancomycin were isolated from bacteria and fungi.[126] Several of them were chemically modified to improve their pharmacological properties during drug development.[127] However, resistance in bacteria to one or multiple antibiotics has been observed over time.[126] Multiple studies established a direct link between the consumption of antibiotics and the rise of resistance in bacteria, making the overuse of antibiotics a major driving factor for this problem.[128,129] Promoting factors for the excessive use of antibiotics are the fact that in some countries antibiotics are unregulated and can be purchased without prescription[130,131] as well as their simplified purchase through the internet.[132,133] Further driving factors for the emergence of drug-resistant bacteria is the incorrectly prescription of antibiotics, their extensive agricultural use and the stalled development of new antibiotics by pharmaceutical companies.[126]

Due to the spread of antibiotic resistance and the resulting infections with multidrug-resistant bacteria that cannot be treated adequately, the Centers for Disease Control and Prevention in the USA declared 18 bacteria and fungi as threats to the public health in 2019. Especially, carbapenem-resistant *Acinetobacter*, *Candida auris*, *Clostridioides difficile*, carbapenem-resistant *Enterobacteriaceae* and drug-resistant *Neisseria gonorrhoeae* were reported as urgent threats that require acute and aggressive action in the USA.[134] Even though there is a growing need for new classes of antibiotics, their development is lagging far behind the demand.[126,135,136] Thus, alternative strategies to fight multidrug-resistant bacteria are warranted.

Many bacteria use quorum sensing (QS), a cell density-dependent mechanism of communication, to measure their population density or sense other species in order to regulate processes, such as bioluminescence,[137,138] biofilm formation,[139,140] siderophore production,[141,142] sporulation,[143,144] motility,[145,146] synthesis of antibiotics,[147–149] and virulence factor expression.[150,151] All known QS systems in bacteria involve releasing, sensing and responding to small molecules, the so-called autoinducers.[152] Many Gram-negative and Gram-positive bacteria produce *N*-acyl-homoserine lactones (**18**, AHLs) and autoinducing peptides, e.g. autoinducing peptide-1 (**19**, AIP-1), as

autoinducers, respectively (**Figure 4**). However, autoinducers from other chemical classes have also been discovered including the *Pseudomonas* quinolone signal (**20**, PQS) in *Pseudomonas aeruginosa*,[153] diffusible signaling factor (**21**, DSF) in *Xanthomonas campestris*,[154] cholera autoinducer-1 (**22**, CAI-1) in *Vibrio cholerae*,[155] and hydroxyl-palmitic acid methyl ester (**23**, PAME) in *Ralstonia solanacearum*.[156] Autoinducer-2 (**24**, AI-2) is another signal molecule produced by the protein LuxS in *Vibrio harveyi*.[157] This protein has been found in over 500 different bacterial species, making **24** the most common autoinducer in bacteria to date, and thus proposed to facilitate interspecies communication.[158] In Gram-negative and Gram-positive bacteria, autoinducers are produced by the cell and diffuse or are secreted into the environment. The autoinducer concentration increases as the bacterial cell density grows. When the autoinducer concentration reaches a certain critical threshold, autoinducers can alter gene expression by binding to transmembrane histidine protein kinase receptors or transcriptional regulators of the cell, allowing the whole population to coordinate its behaviour.[152]

Some pathogens use QS to regulate processes important for their pathogenicity, including biofilm formation, sporulation, and expression of virulence factors, such as haemolysins, adhesins, toxins, siderophores, and proteases.[159] Thus, interference with QS systems in pathogenic bacteria has been discussed as a promising strategy for the treatment of bacterial infections.[160–163] Reduction of pathogenicity in pathogens with the utilization of QS inhibitors could allow the host immune system to clear the infection before it causes too much harm. Various QS inhibitors have been discovered that can interfere with the QS system of bacteria by inhibiting the synthases, degrading the signal molecule, or blocking the response regulators or transcriptional factors.[164]

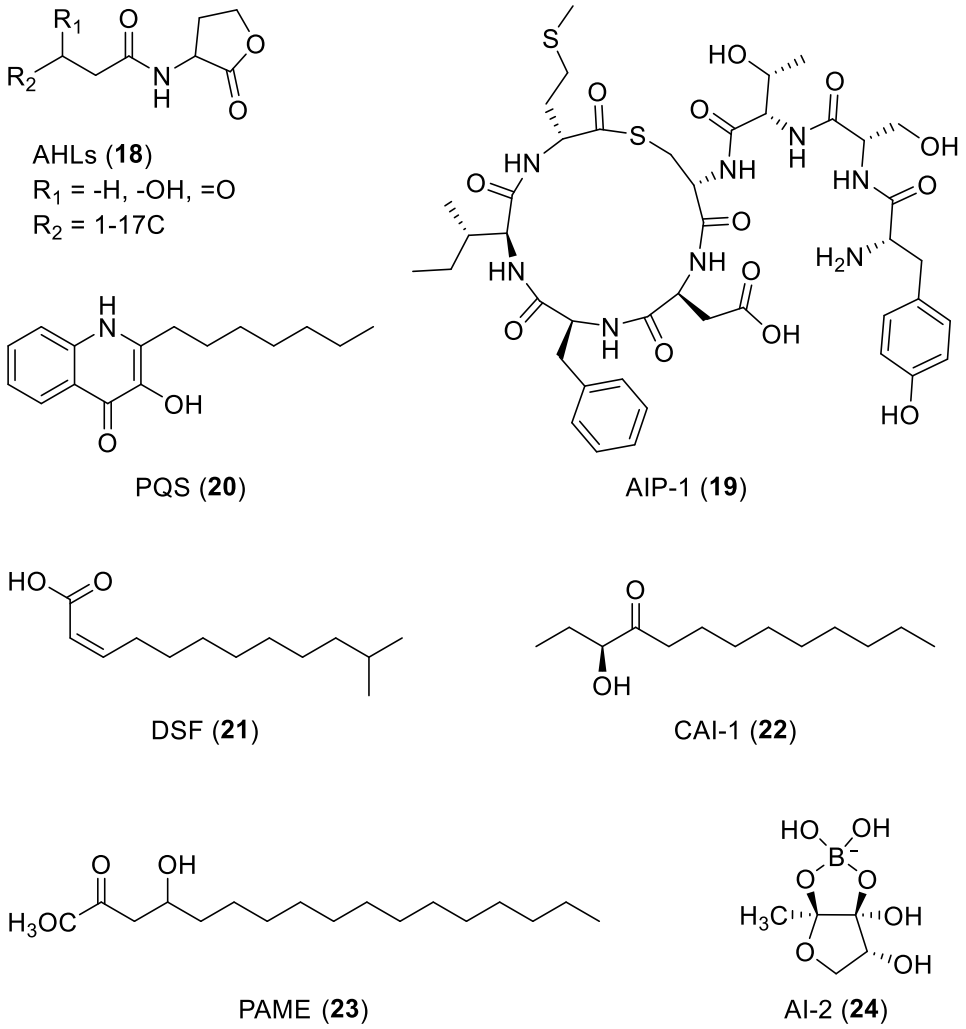
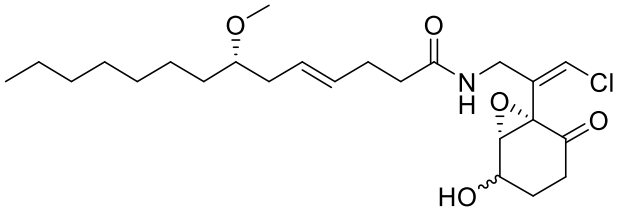


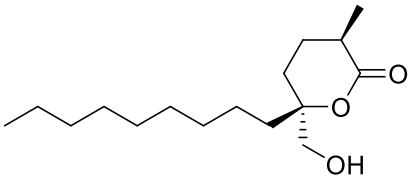
Figure 4. Selected autoinducers from QS systems in different organisms.

Several QS inhibitors that disrupt AHL-based QS systems were isolated from cyanobacteria (**Figure 5**). Extracts from *Lyngbya majuscula* yielded 8-epi-malyngamide C (**25**),[165] malyngamide C (**26**),[165] malyngolide (**27**) and lyngbyoic acid (**28**),[26,166] which showed QS inhibition activity against a LasR-based reporter strain. LasR is a transcriptional regulator and part of the LasI/LasR QS system in *P. aeruginosa*.[167] In addition, **15** and **16** were isolated from *L. majuscula*. Both inhibited the QS of *Chromobacterium violaceum*.[168] Moreover, pitinoic acid A (**29**) was isolated from a marine cyanobacterium morphologically similar to *Lyngbya* sp. as an inhibitor of the QS system in *P. aeruginosa*.[169] Further QS inhibitors were isolated from the cyanobacterium *Blennothrix cantharidosmum* such as tumonoic acids E–H. All showed quorum sensing inhibition activity on the bioluminescence of *V. harveyi*, with tumonoic acid F (**30**) showing the most potent

inhibitory activity.[170] Reduction of the bioluminescence of *V. harveyi* was also attributed to the three γ-butyrolactones, honaucin A–C (**31**–**33**), as well as to lyngbic acid (**34**).[171,172] Honaucins A–C (**31**–**33**) were isolated from the cyanobacterium *Leptolyngbya crossbyana,*[171] whereas **34** was isolated from *L. majuscula*.[26] Although several QS inhibitors have been isolated from cyanobacteria, no QS system has yet been found in this phylum. However, the production of autoinducer *N*-octanoyl-homoserine lactone by a *Gloeothece* sp. strain was reported in 2008. Although the strains showed a QS-like behaviour to the accumulation of *N*-octanoyl-homoserine lactone, no QS genes could be identified in its genome.[173]

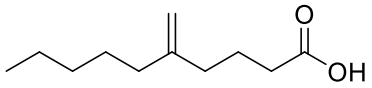


8-epi-malyngamide C (8R; **25**)
Malyngamide C (8S; **26**)

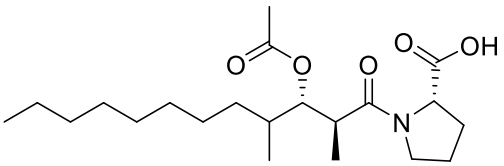


Malyngolide (**27**)

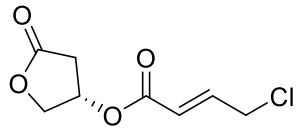
Lyngbyoic acid (**28**)



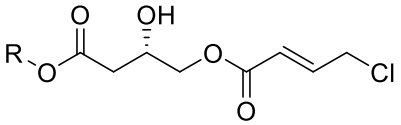
Pitinoic acid A (**29**)



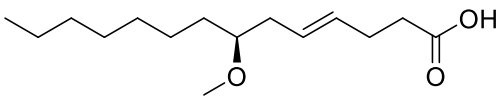
Tumonoic acid F (**30**)



Honaucin A (**31**)



Honaucin B (R = CH_2CH_3; **32**)
Honaucin C (R = CH_3; **33**)



Lyngbic acid (**34**)

Figure 5. Selected natural products from cyanobacteria with QS inhibition activity.

It has been hypothesized that the non-bactericidal nature of QS inhibitors may lead to a lower probability for microorganisms to develop resistance mechanisms against them, suggesting that QS inhibitors are potential alternatives for antibiotics.[174] Another application for QS inhibitors is their combination with antibiotics. Multiple *in vivo* and *in vitro* studies revealed the synergistic effect of QS inhibitors and antibiotics.[175–178] Christensen et al. showed that QS inhibitors furanone C-30, ajoene, or horseradish juice extract provide synergistic antimicrobial effects with tobramycin in combination treatments of *P. aeruginosa* biofilms, making this a promising strategy to improve the efficacy of antibiotics.[176] Currently, no QS inhibitor has been approved as a therapeutic drug. However, several clinical trials have already been performed to assess their potential in treating bacterial infections.[179–181]

1.5 Quorum sensing in *Serratia*

Bacteria from the genus *Serratia* are Gram-negative and belong to the family *Enterobacteriaceae*. *Serratia* species occupy different habitats, such as water, soil, plants, animals, and humans.[182] Most strains are motile by peritrichous flagella, facultative anaerobic and chemoorganotrophic. Several species of *Serratia*, in particular *Serratia marcescens*, are opportunistic pathogens and can cause numerous infections, e.g., pneumonia, bacteremia, wound and urinary tract infections.[183–186] Complications in treatment of *Serratia* infections can occur due to resistance to multiple antibiotics.[187,188]
Similar to other pathogens, the pathogenicity in *Serratia* is controlled among other factors by QS.[189] QS-regulated processes including motility, biofilm formation, and the production of virulence factors that may contribute to the pathogenicity of *Serratia*.[189] The four AHL-dependent QS systems SmaI/SmaR, SwrI/SwrR, SpnI/SpnR, and SplI/SplR were identified in various *Serratia* strains such as *Serratia* sp., *S. marcescens*, *S. plymuthica*, and *S. proteamaculans*, with *N*-hexanoyl-L-homoserine lactone being the most common AHL.[148,190–194] *Serratia* spp. have two forms of flagellum-dependent motility (swarming and swimming) and one flagellum-independent form of motility (sliding). Swarming, which allows the bacterium to move on top of agar surfaces, and the production of molecules that reduce the surface tension of the agar has been shown to be regulated by AHL-dependent QS systems in *Serratia* spp.[192] The production of biofilms has also been reported to be QS-dependent in *Serratia* strains such as in *S. marcescens* MG1 and

S. marcescens strain 12.[195,196] Biofilms are produced by homogenous or heterogeneous bacterial populations that are embedded in extracellular polymeric substances in order to provide advantages to the community, including trapping of nutrients or tolerance to antimicrobials.[197] Moreover, the production of antibiotics like 1-carbapen-2-em-3-carboxylic acid are regulated by QS in *Serratia* sp. ATCC 39006 (S39006).[190] 1-carbapen-2-em-3-carboxylic acid is a broad-spectrum β-lactam antibiotic that inhibits the cell wall biosynthesis in bacteria.[198] The production of extracellular enzymes, such as lipases, proteases, chitinases, nucleases, pectate lyases, and cellulases have also been reported to be QS-regulated in several *Serratia* strains, which may contribute to the their pathogenicity and their ability to spoil food.[190,193,194,196,199]

In addition to the AHL-mediated communication systems, AI-2-dependent QS systems have been discovered in S39006, *S. marcescens* ATCC 274 and *S. marcescens* strain 12.[196,200] Mutants of these strains lacking the ability to produce **24** showed alterations in the production of 1-carbapen-2-em-3-carboxylic acid, prodigiosin, haemolysin and biofilm.[200] Some of the above mentioned processes are not exclusively regulated by QS systems, but are also dependent by other environmental cues, transcriptional or post-transcriptional regulators, such as in the case of prodigiosin.[201]

1.6 Prodigiosin production in *Serratia*

Serratia species such as *S. marcescens*, *S. rubideae* and *S. plymuthica* produce a red pigment called prodigiosin.[182] It belongs to the chemical family of prodiginines that can be classified into four structural classes. Prodigiosin (**35**) and undecylprodigiosin (**36**) belong to the class of linear prodiginines, whereas butyl-meta-cyclo-heptylprodigiosin (**37**), cylcoprodigiosin (**38**), and cyclononylprodigiosin (**39**) represent three other classes (**Figure 6**).[202] Prodiginines have been isolated from *Alteromonas*, *Beneckea*, *Hahella*, *Pseudoalteromonas*, *Pseudomonas*, *Serratia*, *Vibrio* and various actinobacteria such as *Streptomyces*, *Streptoverticillium*, *Actinomadura*, and *Saccharopolyspora*.[202]

Various studies showed that prodiginines exhibit antibacterial, antiprotozoal, antifungal, and antimalarial activities.[203–206] Moreover, due to their potent anticancer and immunosuppressive activities, they have a great therapeutic potential in clinical use.[207] A synthetic compound, obatoclax, that is based on a prodigiosin scaffold has already been

tested in phase I and II clinical trials for the treatment of acute myeloid leukemia, small cell lung cancer, Hodgkin's lymphoma, and myelodysplastic syndromes.[208–211]

The physiological role of prodigiosin in the producer organisms is still debated. Prodigiosin was proposed as a "metabolic sink" for bacteria to catabolize metabolites from primary metabolism, including proline.[212] Moreover, it was suggested that prodigiosin may be an important factor in surface adherence, bacterial dispersion, and the trypanolytic activity of *Serratia*.[213–215] However, it is assumed that prodigiosin production and its regulation by QS is not essential for the proliferation of *S. marcescens*, since corresponding genes are missing in many *S. marcescens* strains.[216]

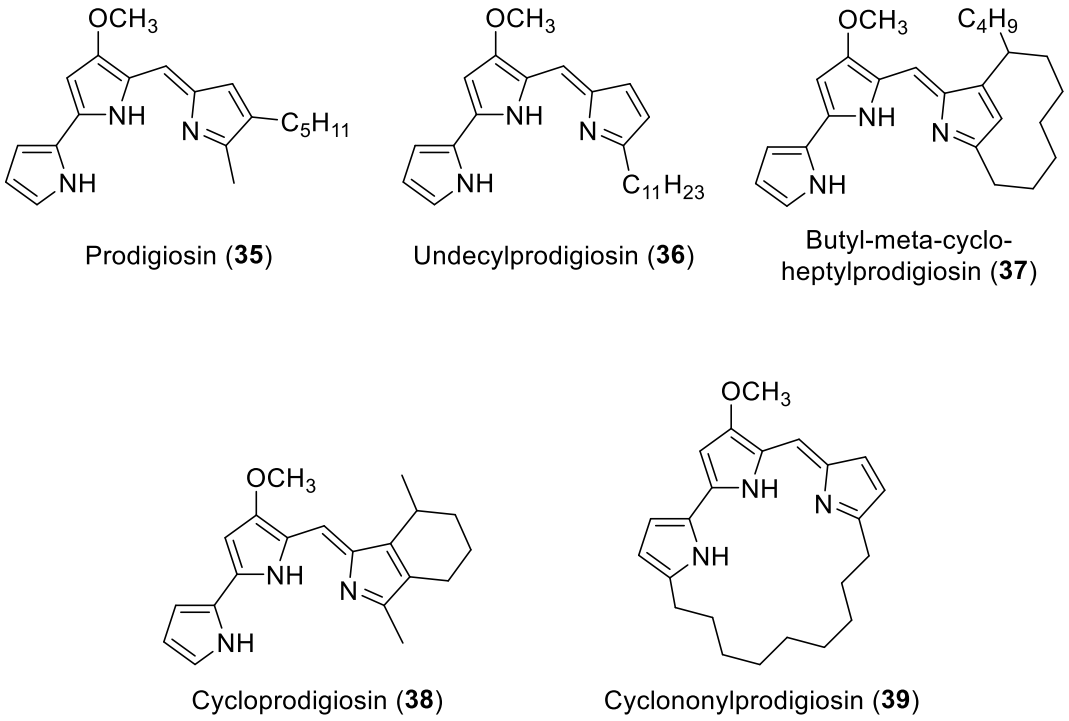


Figure 6. Selected natural products from the prodiginine family.

Prodigiosin is produced by a combination of PKS and NRPS enzymes that are encoded in the biosynthetic gene cluster *pig*. In *S. marcescens* ATCC 274 the *pig* cluster is comprised of 14 genes (*pigA-N*). In contrast, the *pig* cluster in S39006 consists of 15 genes (*pigA-O*), due to one additional gene (*pigO*) that has probably a regulatory function.[217]

The production of prodigiosin is facilitated by a bifurcated pathway (**Figure 7**). One branch of the pathway synthesizes 2-methyl-3-n-amyl-pyrrole (**44**) with the enzymes PigD, PigE, and PigB. It is assumed that PigD transfers an acetyl group from pyruvate (**40**) on *trans*-2-octenal (**41**), resulting in the formation of (*S*)-3-acetyloctanal (**42**).[218] Subsequently, PigE performs a transamination on the aldehyde group of **42**, leading to a spontaneous aminoketone cyclization and the formation of 2-methyl-3-pentyl-4,5-dihydro-3H-pyrrole (**43**).[218] The last step is catalyzed by PigB with the oxidation of **43** to **44**.[218]

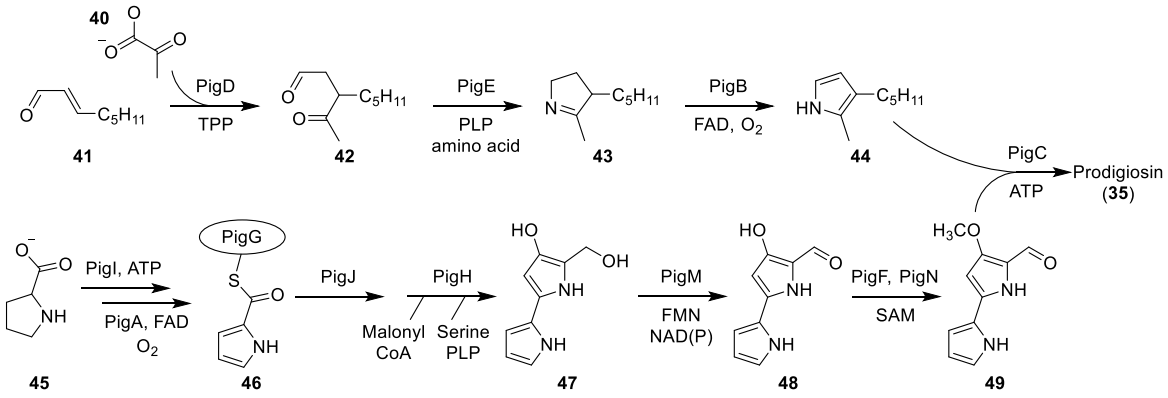


Figure 7. The biosynthetic pathway of prodigiosin in *Serratia* sp. ATCC 39006, adapted from Couturier et al.[219] TPP, thiamine pyrophosphate; PLP, pyridoxal phosphate; FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide; NAD(P), nicotinamide adenine dinucleotide phosphate; SAM, S-adenosyl-L-methionine; ATP, adenosine triphosphate.

The second branch of the biosynthesis facilitates the formation of 4-methoxy-2,2'-bipyrrole-5-carbaldehyde (**49**) using the enzymes PigI, PigG, PigJ, PigH, PigM, PigF, and PigN. The enzyme PigI enables the ligation of L-proline (**45**) to the PigG acyl-carrier protein, following with the oxidation of L-prolyl-S-PCP to pyrrolyl-2-carboxyl-S-PCP (**46**) by PigA.[217,220] Then, the pyrrole-2-carboxyl unit from PigG is transferred to PigJ, whereas a malonyl group from Malonyl-CoA is attached to PigH. Subsequently, L-serine is decarboxylated, resulting in the attack of the malonly unit on the pyrrole-2-carboxyl

thioester. The decarboxylative attack leads to the release of the pyrrole-2-carboxyl unit from PigG and its formation to pyrrolyl-β-ketothioester connected to PigH.[218] It is assumed that PigH condenses pyrrolyl-β-ketothioester and serine to 4-hydroxy-2,2′-bipyrrole-5-methanol (**47**). A following oxidation of the alcohol group of **47** by PigM results in the intermediate 4-hydroxy-2,2′-bipyrrole-5-carbaldehyde (**48**). Consecutively, PigF/PigN catalyze the methylation of **48** yielding the intermediate **49**. In a final reaction, PigC catalyzes the condensation of the two intermediates from both branches, **44** and **49**, resulting in the final product prodigiosin.[218]

1.7 Regulation of prodigiosin production in S39006

Biosynthesis of prodigiosin in S39006 is controlled by a complex regulatory network that is dependent on QS, transcriptional and post-transcriptional regulators, as well as environmental cues (**Figure 8**). The QS system in S39006 is comprised by SmaI and SmaR.[190] The synthase SmaI produces *N*-butanoyl-L-homoserine lactone and *N*-hexanoyl-L-homoserine lactone as autoinducers.[190] SmaR is a transcriptional regulator that represses the transcription of the *pig* cluster as well as additional transcriptional regulators (PigQ, Rap, PigR) at low cell densities.[221] The increase of cell density leads to accumulation of AHLs that can bind to SmaR and attenuate its repressive activity. Therefore, the highest production of prodigiosin can be observed at high cell densities, such as in the late exponential phase and stationary phase of the bacterial population.[221]

A central role in the regulatory network of prodigiosin production play the transcriptional regulators PigP, FloR and SmaR.[221,222] All three transcriptional regulators control directly or indirectly the transcription of multiple transcriptional and post-transcriptional regulators, including activators (FlhDC, PhoBR, PigQ, PigR, PigS, PigT, PstSCAB-PhoU, Rap, SdhE, YgfX) and repressors (PigU, PigX, RpoS, RsmAB) of prodigiosin production.[201,223–227] In contrast to FloR, PigP and SmaR also regulate the PIG production directly by altering the expression of the *pig* cluster. PigZ is another transcriptional repressor belonging to the TetR family. However, this regulator is not influenced by PigP, FloR, or SmaR.[228]

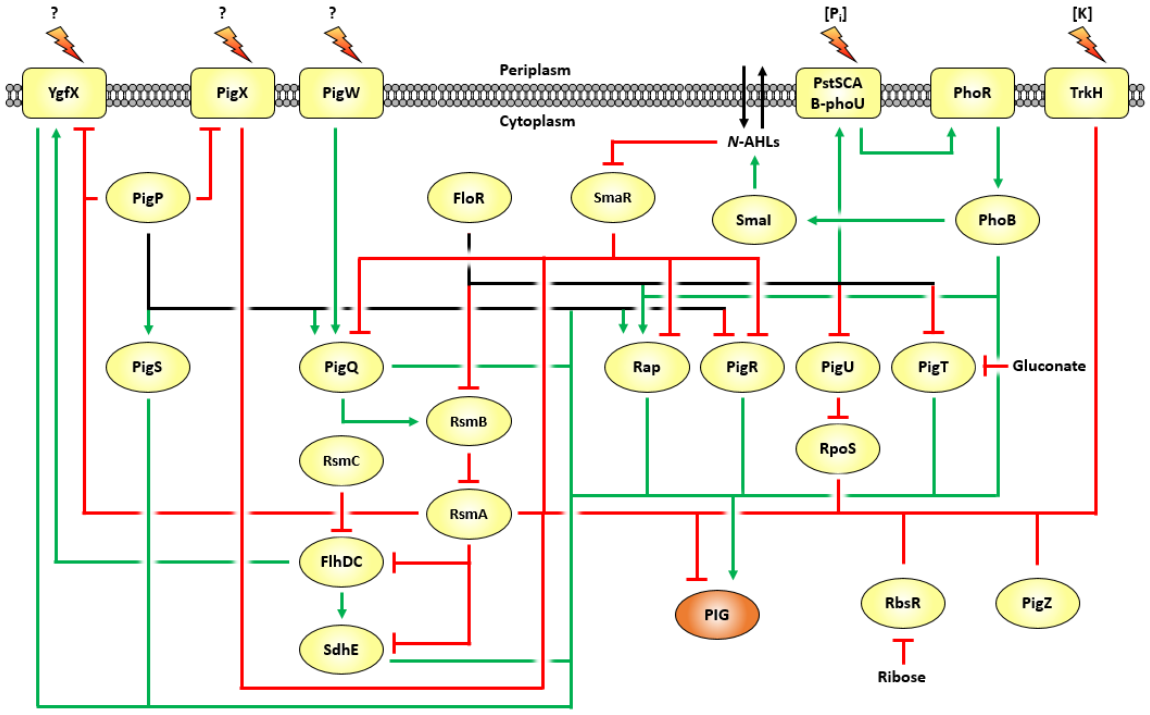


Figure 8. Model of the regulatory network controlling prodigiosin (PIG) production in S39006, adapted from Williamson et al.[201] Activation is represented by green arrows, repression is displayed by red lines with flat arrow-heads. Detailed information for the individual pathways is described in the text.

Moreover, several environmental cues, including AHLs, inorganic phosphate, potassium and various carbon sources, such as gluconate and ribose, affect production of prodigiosin in S39006. The detection of inorganic phosphate is facilitated by the high affinity phosphate transporter PstSCAB-phoU and the two-component system PhoBR. At low inorganic phosphate concentrations, PstSCAB-phoU induces PhoR, resulting in the activation of PhoB by phosphorylation, which in turn activates the expression of *smaI* and the *pig* cluster.[229,230] Most recently, it has been shown that the potassium transporter TrkH modulates prodigiosin production in S39006. High potassium concentrations reduced production of prodigiosin in this strain.[231] Another two-component system in S39006 that is involved in the regulation of prodigiosin consists of PigW and PigQ. PigW is a membrane-bound protein that is autophosphorylated in response to an unknown environmental input. Due to activation, PigW phosphorylates the response regulator PigQ, resulting in the activation of the post-transcriptional regulator RsmB and indirectly in the activation of prodigiosin production.[232] The membrane bound proteins YgfX and PigX

represent further regulatory proteins.[221] However, their environmental inputs are not known to date. PigX might possess a cyclic-di-GMP-specific diguanylate cyclase and phosphodiesterase activities. Thus, intracellular cyclic-di-GMP could potentially be the environmental cue of PigX.[233] Furthermore, ribose is an effector of the LacI-family transcription factor RbsR. This protein controls prodigiosin production as well as other physiological processes, such as the production of gas vesicles, siderophore, cellulose, and swimming and swarming motility.[234] Gluconate is another carbon source that regulates prodigiosin production in S39006. It has been shown that the addition of gluconate to a S39006 culture represses the transcriptional activator PigT, resulting in reduced prodigiosin production.[235]

2 Research aim and objectives

Since ancient times natural products have been widely used to treat diseases, and they continue to play an important role in the discovery of new drugs and drug leads. Cyanobacteria have been recognized as a prolific source for new bioactive natural products. Thus, this thesis aimed to identify and characterize new natural products from cyanobacteria for drug development purposes. Three different approaches were used to achieve this goal: (I) a chemistry-guided screening was performed with the software tool HaloSeeker in an effort to identify novel HNPs from cyanobacteria; two distinct bioassay-based screening campaigns were conducted to identify (II) immunomodulating natural products as well as (III) QS inhibitors targeting the QS system of *S. marcescens*. New natural products identified by the screenings were isolated and their structures elucidated. Bioactivities of the isolated compounds were characterized in order to evaluate their potential as candidates for drug development or other applications. The results of all three research objectives were published as original papers and are summarized in the following sections.

3 Summary of results

3.1 Publication 1: "Halogenation-guided chemical screening provides insight into tjipanazole biosynthesis by the cyanobacterium *Fischerella ambigua*"

Tomasz Chilczuk, Till F. Schäberle, Sahel Vahdati, Ute Mettal, Mustafa El Omari, Heike Enke, Michael Wiese, Gabriele M. König, Timo H. J. Niedermeyer
ChemBioChem 2020, *21*, 2170–2177, DOI: 10.1002/cbic.202000025
The Supporting Information is available at
https://chemistry-europe.onlinelibrary.wiley.com/doi/full/10.1002/cbic.202000025.

HNPs exhibit various bioactivities including antibacterial, antifungal, antiparasitic, antiviral, anti-inflammatory, antitumor and molluscicidal activities that can be utilized for the development of new drugs and drug leads. Thus, the aim of this study was to identify and characterize new HNPs from cyanobacteria. In order to achieve this aim, a chemistry-guided screening with the software tool HaloSeeker dedicated to identifying halogenated compounds was conducted. The analysis of the HPLC-HRMS data of a *F. ambigua* (Näg.) Gomont 108b extract with HaloSeeker indicated the existence of several unknown HNPs. Thus, five HNPs were isolated using flash chromatography and semi-preparative HPLC. The structures of all isolated compounds were elucidated based on 1D, 2D and HRMS data. All isolated compounds belong to the chemical family referred to as tjipanazoles. Two compounds were dereplicated as tjipanazole D and I, whereas the structures of the remaining three compounds are new to nature. The new tjipanazoles were named tjipanazole K, L, and M.

Taking into account previous work on tjipanazole biosynthesis, the reported biosynthetic pathways for the structurally related natural products rebeccamycin and staurosporine, and based on a bioinformatics analysis of the *F. ambigua* (Näg.) Gomont 108b genome sequence and the tjipanazoles detected in the strain, we proposed a putative biosynthetic pathway for the tjipanazoles. The tjipanazole biosynthetic gene cluster is comprised of ten genes (*tjp1-10*). The enzymes Tjp1, Tjp2, Tjp7, Tjp8, and Tjp10 show homology to enzymes from the rebeccamycin and staurosporine biosynthetic pathway and catalyze possibly similar reactions leading to the synthesis of tjipanazole K, J, L, and M.

The remaining enzymes Tjp3–6 are expected to be involved in the removal of the pyrrolo[3,4-c] ring, resulting in the production of tjipanazole D and I.

ABCG2 is a clinically relevant multidrug-transporter overexpressed by different cancer cell lines and is known to contribute to multidrug resistance in cancer by affecting the pharmacokinetics of anticancer drugs. K252c and arcyriaflavin A showed inhibitory activity against the ABCG2 transporter and share structural similarities with the isolated tjipanazoles. Thus, all tjipanazoles were tested for inhibitory activity against ABCG2. Tjipanazole M showed the highest inhibitory activity against ABCG2 from all tjipanazoles with IC_{50} values comparable to arcyriaflavin A. The performed structure-activity relationship study showed that the loss of the pyrrolo[3,4-c] ring as well as the presence of two chlorine substituents decreases inhibitory activity against ABCG2. In contrast, the exchange of a carbonyl group with a methylene group at the pyrrolo[3,4-c] ring increases the inhibitory activity of the tested compounds.

Halogenation-Guided Chemical Screening Provides Insight into Tjipanazole Biosynthesis by the Cyanobacterium *Fischerella ambigua*

Tomasz Chilczuk,[a] Till F. Schäberle,[b, f] Sahel Vahdati,[c] Ute Mettal,[b, f] Mustafa El Omari,[d] Heike Enke,[e] Michael Wiese,[c] Gabriele M. König,[d] and Timo H. J. Niedermeyer*[a]

Halogenated natural products (HNPs) show a wide range of interesting biological activities. Chemistry-guided screening with a software tool dedicated to identifying halogenated compounds in HPLC-MS data indicated the presence of several uncharacterised HNPs in an extract of the cyanobacterium *Fischerella ambigua* (Näg.) Gomont 108b. Three new natural products, tjipanazoles K, L, and M, were isolated from this strain together with the known tjipanazoles D and I. Taking into account the structures of all tjipanazole derivatives detected in this strain, reanalysis of the tjipanazole biosynthetic gene cluster allowed us to propose a biosynthetic pathway for the tjipanazoles. As the isolated tjipanazoles show structural similarity to arcyriaflavin A, an inhibitor of the clinically relevant multidrug-transporter ABCG2 overexpressed by different cancer cell lines, the isolated compounds were tested for ABCG2 inhibitory activity. Only tjipanazole K showed appreciable transporter inhibition, whereas the compounds lacking the pyrrolo[3,4-c] ring or featuring additional chloro substituents were found to be much less active.

Introduction

More than 5000 halogenated natural products (HNPs) are known today, and the number is constantly growing.[1] HNPs constitute a diverse group of metabolites with a wide range of bioactivities, including antibacterial, antifungal, antiparasitic, antiviral, anti-inflammatory and antitumor activities.[2] Some of these HNPs are used as antibiotics (e.g., vancomycin,[3] chloramphenicol[4]) or are studied as potential antitumor agents (e.g., rebeccamycin[5], cryptophycins[6]). The presence of halogen substituents often enhances the bioactivity of natural products,[7] as exemplified by vancomycin, where mono- and didechlorovancomycin show two to four times lower activity in binding to aliphatic peptides than vancomycin.[8] Halogenation is also a widely used strategy to modify the activity of small synthetic drug molecules.[9]

The majority of known HNPs are biosynthesised by marine bacteria,[10,11] marine algae[10,12] and marine invertebrates.[13] However, their production has been also reported in terrestrial plants,[14] fungi,[15] soil bacteria,[3,16] insects,[17] and higher animals.[18] The search for HNPs from cyanobacteria is particularly interesting because cyanobacteria are considered to be a rich source of bioactive and chemically diverse compounds,[19] and are well known to synthesise HNPs, such as aeruginosins,[20] cyanopeptolins,[21] cryptophycins,[22] microginins,[23] lyngbyabellins,[24] and the indole alkaloids ambiguines,[25] hapalindoles,[26] welwitindolinones,[27] and fischerindoles.[28] Other cyanobacterial HNPs from various chemical classes are the ambigols,[29] bartolosides,[30] carbamidocyclophanes,[31] malyngamides,[32] and tjipanazoles.[33]

In an effort to identify novel HNPs from cyanobacteria, a chemistry-guided screening was conducted with the software tool HaloSeeker.[34] In full-scan HPLC-HRMS data of an extract of *Fischerella ambigua* (Näg.) Gomont 108b, we could detect far more polyhalogenated compounds than described from this strain to date. As currently only 23 HNPs from the genus *Fischerella* are listed in the Dictionary of Natural Products (18 indole alkaloids, 3 ambigols, 2,4-Dichlorobenzoic acid, and tjipanazole D),[29,35,36] this suggested the strain to be a promising

[a] *T. Chilczuk, Prof. Dr. T. H. J. Niedermeyer*
Department of Pharmaceutical Biology/Pharmacognosy
Institute of Pharmacy
Martin-Luther-University Halle-Wittenberg
Hoher Weg 8, 06120 Halle (Saale) (Germany)
E-mail: timo.niedermeyer@pharmazie.uni-halle.de
[b] *Prof. Dr. T. F. Schäberle, Dr. U. Mettal*
Institute for Insect Biotechnology
Justus-Liebig-University Gießen
Heinrich-Buff-Ring 26–32, 35392 Gießen (Germany)
[c] *S. Vahdati, Prof. Dr. M. Wiese*
Department of Pharmaceutical and Cell Biological Chemistry
University of Bonn
An der Immenburg 4, 53121 Bonn (Germany)
[d] *Dr. M. El Omari, Prof. Dr. G. M. König*
Institute of Pharmaceutical Biology, University of Bonn
Nussallee 6, 53115 Bonn (Germany)
[e] *Dr. H. Enke*
Cyano Biotech GmbH
Magnusstraße 11, 12489 Berlin (Germany)
[f] *Prof. Dr. T. F. Schäberle, Dr. U. Mettal*
Department of Bioresources
Fraunhofer Institute for Molecular Biology and Applied Ecology
Winchesterstrasse 2, 35394 Gießen (Germany)

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbic.202000025

This article is part of a Special Collection on Microbial Biosynthesis and Interactions. To view the complete collection, visit our homepage

source for new HNPs. Subsequent isolation of some of the HNPs detected in this strain led to the identification of the new natural products tjipanazoles K (**3**), L (**4**) and M (**5**), along with the known tjipanazoles D (**1**) and I (**2**). Their structures were elucidated using 1D and 2D NMR and HRMS.

Tjipanazole D has been proposed to be synthesised from two L-tryptophans by five enzymes, an L-tryptophan halogenase, an L-tryptophan oxidase, a chromopyrrolic acid synthase-like protein, a CYP 450 enzyme, and a FAD-binding monooxygenase. The first two enzymes are supposed to catalyse the conversion of L-tryptophan to 5-chloro-indole-3-pyruvic acid (chlorination and oxidative desamination), whereas the latter three enzymes use this intermediate in subsequent reactions (dimerisation, aryl-aryl-coupling, side-chain reduction, aromatisation) for the assembly of tjipanazole D.[37] The structures of the three new tjipanazole derivatives and the detectable halogenated intermediates of the tjipanazole biosynthesis, as well as a more detailed analysis of the genome of this *F. ambigua* strain, allowed us to revisit and substantiate the formerly described proposed putative tjipanazole biosynthesis pathway.

All three new tjipanazoles share an indolocarbazole core structure and show structural similarities to the ABCG2 inhibitors K252c (**7**) and arcyriaflavin A (**8**).[38] ABCG2, also known as breast cancer resistance protein, is an ATP-binding cassette transporter that protects many tissues against xenobiotics by transporting them across extracellular and intracellular membranes. It is known to contribute to multidrug resistance in cancer by affecting the pharmacokinetics of anticancer drugs.[39] Due to the clinical relevance of ABCG2-mediated multidrug chemotherapy resistance, extensive efforts have been devoted to the development of ABCG2 inhibitors that could be used in combination with anticancer drugs to reduce their secretion from cancer cells.[40] Based on the studies of Rubey et al.,[38] ABCG2 is regarded as a promising new target for indolocarbazoles. Thus, all isolated compounds were tested for their capability to inhibit ABCG2.

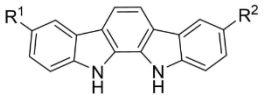
Results and Discussion

Isolation and structure elucidation

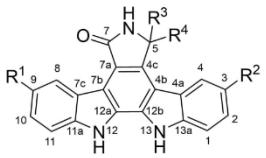
In a chemistry-guided screening for HNPs in cyanobacteria extracts using HaloSeeker, a post-acquisition processing tool designed to annotate signals from halogenated ions within HRMS data sets, an extract of *F. ambigua* (Näg.) Gomont 108b was found to be rich in HNPs. Evaluation of the HRMS data resulted in the detection of 105 analyte species with isotope patterns typical for halogenated substances (Figure S1 in the Supporting Information). Fractionation of the biomass extract of this strain using flash chromatography resulted in 24 fractions. The fractions containing the major HNPs were combined.

Several compounds were isolated from the combined fraction using semi-preparative HPLC, among them compounds **1**–**5**. The UV spectra of the compounds suggested that they are structurally related (Figure S2). The molecular formulas were deduced from HRMS data, the structures were elucidated based on 1D and 2D NMR (Figure 1). The NMR and HRMS data of **1** and **2** matched the published data of tjipanazole D and I, respectively (Figures S3–S5).[33] Tjipanazole D (**1**) has been isolated from the same strain before,[29,36,41] whereas tjipanazole I (**2**) has already been reported as a natural product from the cyanobacterium *Tolypothrix tjipanasensis*.[33]

Compound **3** was isolated as yellow crystals. Its molecular formula was established as $C_{20}H_{11}Cl_2N_3O$ by the $[M-H]^-$ ion at *m/z* 378.0207 (calcd. 378.0206, Δ 0.01 ppm). Compared to **1**, the sum formula of **3** indicated the presence of an additional nitrogen as well as two carbon atoms, suggesting it to be more closely related to tjipanazoles containing an additional pyrrolo substructure. Indeed, the mass difference between **3** and the known tjipanazole J (**6**) was 16 Da, suggesting **3** being a tjipanazole J derivative missing one oxygen. The ^{1}H NMR data of **3** showed close similarities to those of **6** (Figures S6–S8).[33] However, H-5 of **3** (δ_H 4.98 ppm) is stronger shielded than the respective proton of **6** (δ_H 6.42 ppm), suggesting the lack of the hydroxy group at C-5. The position of the methylene group at the pyrrolo[3,4-c] ring was further confirmed comparing the NMR data of **3** with those of the staurosporine aglycone K252c (**7**). Compound **7** is an indolo[2,3-a]pyrrolo[3,4-c]carbazole with-



1 R^1 = Cl; R^2 = Cl
2 R^1 = Cl; R^2 = H



3 R^1 = Cl; R^2 = Cl; R^3 = R^4 = H
4 R^1 = Cl; R^2 = Cl; R^3, R^4 = O
5 R^1 = Cl; R^2 = H; R^3, R^4 = O
6 R^1 = Cl; R^2 = Cl; R^3 = H, R^4 = OH
7 R^1 = H; R^2 = H; R^3 = R^4 = H
8 R^1 = H; R^2 = H; R^3, R^4 = O

Figure 1. Chemical structures of tjipanazoles **1**–**5** isolated from *F. ambigua*, tjipanazole J (**6**), K252c (**7**), and arcyriaflavin A (**8**).

out chlorine substituents. The ^{1}H and ^{13}C chemical shifts of the pyrrolo substructures of **3** and **7** were in good agreement, confirming the methylene group at position C-5.[42] Thus, **3** was identified as the new natural product tjipanazole K. Compound **3** has already been reported as synthetic substance, and has been tested for protein kinase C (PKC) inhibition and treatment of AIDS.[43]

Compound **4** was isolated as yellow crystals. Its molecular formula was established as $C_{20}H_9Cl_2N_3O_2$ by the $[M\text{-}H]^-$ ion at *m/z* 391.9997 (calcd. 391.9999, Δ 0.49 ppm), two protons less than **6**. The ^{1}H spectrum of **4** showed just three signals for aromatic protons (δ_H 8.96, 7.81, 7.56 ppm) in addition to two NH protons (δ_H 12.98, 11.11 ppm), thus indicating a symmetrical molecule. Due to the obvious symmetry of **4**, both protons can only be missing at the pyrrolo[3,4-c] ring, resulting in an indolo [2,3-a]pyrrolo[3,4-c]carbazole skeleton with two carbonyl groups for **4**. This was confirmed by 2D NMR experiments (HSQC-DEPT, HMBC; Figures S9–S12). Compound **4** has already been described as a synthetic substance.[44,45] NMR data of **4** were in good agreement with the published data, thus **4** was identified as the new natural product tjipanazole L.

Compound **5** was isolated as yellow crystals. Its molecular formula was established as $C_{20}H_{10}ClN_3O_2$ by the $[M-H]^-$ ion at *m/z* 358.0390 (calcd. 358.0389, Δ 0.46 ppm), one chlorine atom less than **4**. Indeed, the ^{1}H spectrum of **5** showed seven aromatic protons, whereas in **3** and **4**, only six aromatic protons can be observed. The splitting patterns in the ^{1}H spectrum showed the presence of one 1,2,4-trisubstituted and one 1,2-disubstituted benzene ring (Figure S13–S15), confirming the structure of the new natural product tjipanazole M (**5**). Compound **5** has already been described as a substance resulting from a combinatorial biosynthesis study being produced by a recombinant strain of *Streptomyces albus*.[46] The ^{1}H chemical shifts from this publication are in good agreement with our ^{1}H NMR data. However, taking into consideration our 2D NMR data of **3**, **4**, and **5**, as well as the published NMR data of other indolo[2,3-a]pyrrolo[3,4-c]carbazoles, we reassign the ^{13}C chemical shifts for **5** as given below.

Tjipanazole biosynthesis

Over the years, hundreds of bisindole alkaloids have been isolated from natural sources. The structures of the respective biosynthetic precursors, two L-tryptophans, are usually easily recognisable in the final structure. Taking into account previous work on tjipanazole biosynthesis,[37] the reported biosynthetic pathways for the structurally related natural products rebeccamycin and staurosporine,[46,47] based on a bioinformatic analysis of the *F. ambigua* (Näg.) Gomont 108b genome sequence and the tjipanazoles detected in the strain, we propose the biosynthesis pathway of the tjipanazoles as follows (Figure 2B):

In analogy to the biosynthesis of rebeccamycin, halogenation of tryptophan is most likely the first step in the tjipanazole biosynthesis. This reaction is catalysed by the putative $FADH_2$-dependent tryptophan halogenase Tjp10. Subsequently, the monoamine oxidase Tjp1 converts 5-chloro-tryptophan to 5-chloro-indole-3-pyruvate imine. Then, Tjp2, showing homology to previously described chromopyrrolic acid synthases, installs a carbon-carbon bond between the β-carbon atoms of the two indole-pyruvate imines, thereby generating a dimer. As we found that both mono- and dichlorinated tjipanazoles are produced by the strain, the presence of a chlorine atom at C-3 does not seem to be essential for substrate recognition.

Tjp9 is a cytochrome P450 enzyme showing homology to RebP and StaP. This oxygenase can be expected to react with the chromopyrrolic acid-like intermediate. The catalytic action of the cyp450 enzyme enables an aryl-aryl coupling between the two C-2 carbons of the indole systems. A mechanism via the generation of two indole cation radicals is supported by the crystal structure of the homologues StaP.[48] Consecutively, the FAD-binding monooxygenases Tjp7 and/or Tjp8 could catalyse the modification of the pyrrole substructure, comparable to the reaction catalysed by the homologous proteins RebC and StaC (Figure 2A, Table S1). Interestingly, Tjp7, which is only 251 amino acids in size, shows homology with the N-terminal half of RebC/StaC proteins (~550 amino acids), whereas the 314 amino acids of Tjp8 show homology to the C-terminal part of RebC/StaC. We assume that Tjp7 and Tjp8 together form a multifunctional enzyme that is able to modify the pyrrole substructure in various degrees, resulting in multiple products, tjipanazole K (**3**), J (**6**), L (**4**) and M (**5**).

In contrast to staurosporine and rebeccamycin, some tjipanazoles, that is, tjipanazole D (**1**) and I (**2**), lack the pyrrolo [3,4-c] ring, which is cleaved during biosynthesis. One possibility for this cleavage could be that the decarboxylation and hydrolysis reactions take place prior to the formation of the pyrrole ring (Figure S16). The second C–C bond between the indole rings might then be formed in the dimeric intermediate to finally yield an aromatic ring connecting the tryptophan units. The accessory atoms might finally be cleaved off by decarboxylation, hydrolysis and oxidation reactions to give the tjipanazoles D (**1**) and I (**2**). Alternatively, the oxidative degradation of tjipanazoles might start from the hemiaminal tjipanazole J (Figure 2B). We think that the latter (removal of the pyrrolo[3,4-c] ring) is likely the pathway used for the biosynthesis of tjipanazoles D (**1**) and I (**2**), as we could detect almost all intermediates of the latter proposed pathway as $[M-H]^-$ ions in the extract of *F. ambigua* (Figures 2B, S17 and S18), while no intermediates of the other potential pathway were detected.

The genes encoding for the enzymes Tjp3-6 have no counterparts in the rebeccamycin and/or staurosporine biosynthetic gene clusters (BGCs, Figure 2A, Table S1). From the producer organisms harbouring these gene clusters, no alkaloids without the pyrrolo ring are reported. Therefore, involvement of Tjp3-6 in the cleavage of this pyrrolo substructure can be expected. The detailed investigation of these enzymatic steps during tjipanazole biosynthesis will be subject to further studies.

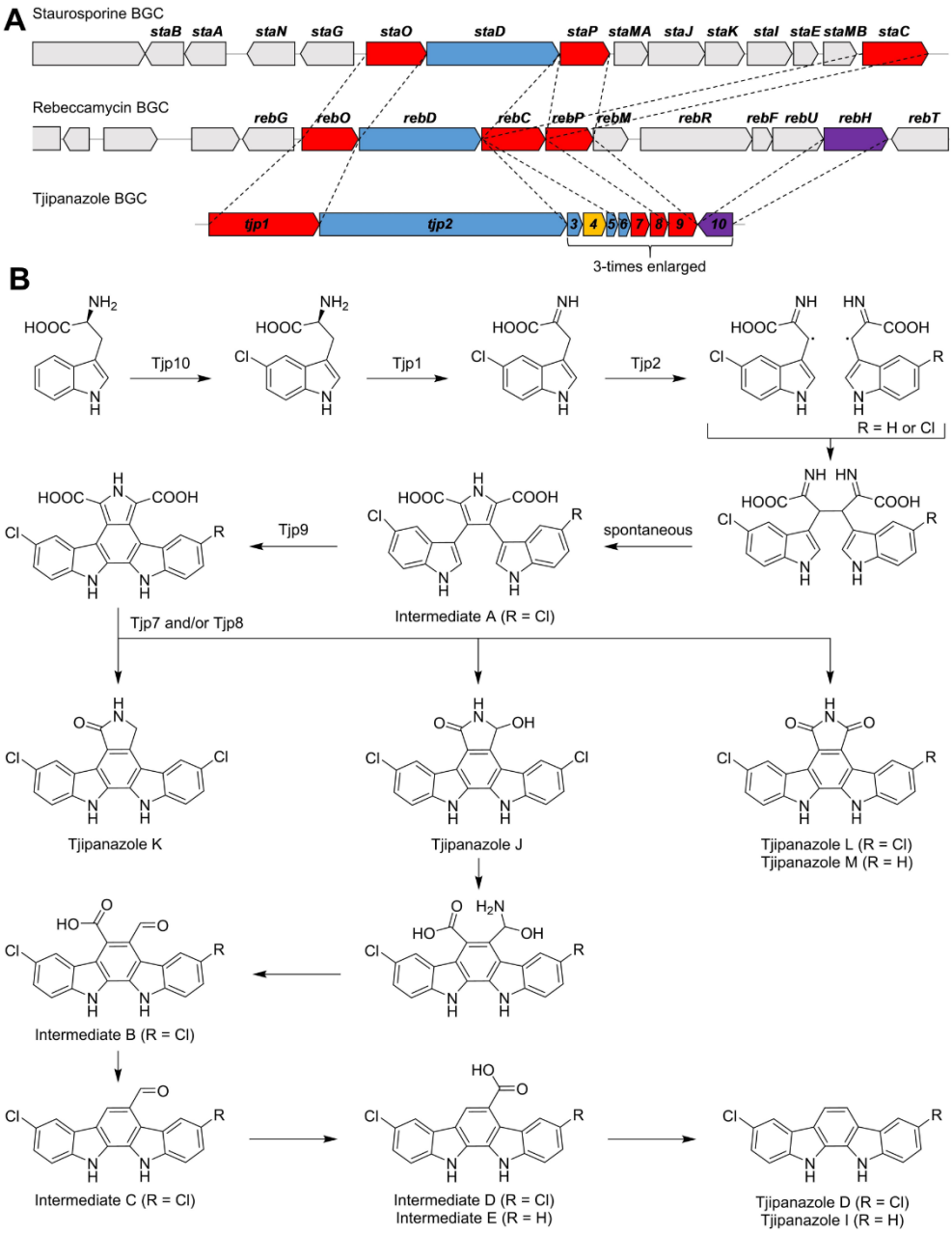


Figure 2. A) Comparison of the putative biosynthetic gene cluster (BGC) of tjipanazole biosynthesis in *F. ambigua* (Näg.) Gomont 108b with the BGC of staurosporine in *Streptomyces* sp. TP-A0274 and the BGC of rebeccamycin in *Lechevalieria aerocolonigenes* ATCC 39243. Oxidase-like genes are coloured red, the methyltransferase-like gene in yellow, halogenase-like genes in violet, and the remaining in blue. Genes are drawn to scale, tjp3–10 are enlarged. Dashed lines show homology of the genes between the BGCs (for details concerning the homologous genes, see Table S1). B) Putative biosynthetic pathway of the tjipanazoles in *F. ambigua* (Näg.) Gomont 108b. All named metabolites were detected by HPLC-HRMS as $[M - H]^-$ ions in the extract of the strain (R = Cl or H). For the intermediates, the position of the functional groups has not been experimentally proven.

Bioactivity of tjipanazoles

Tjipanazoles A1 and A2 showed *in vitro* antifungal activity against rice blast and leaf rust wheat infections, as well as weak, non-selective cytotoxicity against leukaemia and solid tumour cell lines. Protein kinase C was not inhibited.[33] Tjipanazole D exhibited moderate antibacterial activity against Gram-positive bacteria, whereas no antialgal, antifungal, antiparasitic, molluscicidal, or cytotoxic activity was found.[36,49]

ABCG2, an ATP-binding cassette transporter with importance in drug resistance, was identified as target of indolocarbazoles such as K252c (**7**) and arcyriaflavin A (**8**). Both **7** and **8** showed inhibitory activity against the ABCG2-mediated efflux of pheophorbide A.[38,50] Due to structural similarities of the tjipanazoles to **7** and **8**, compounds **1–5** and **8** were tested for inhibitory activity against ABCG2 (Figure 3).

Tjipanazole M (**5**) and arcyriaflavin A (**8**) showed the highest inhibitory activity against ABCG2 from all tested compounds with an IC_{50} of 11 and 9 μM, respectively. Weaker activity was found for tjipanazoles I (**2**, 35 μM), D (**1**, 43 μM), and K (**3**, 75 μM). Tjipanazole L (**4**, estimated 100 μM) was the least active compound. Based on these results, the following structure-activity relationships could be deduced: I) the loss of the pyrrolo[3,4-c] ring as well as II) the presence of two chlorine substituents at position C-3 and C-9 decreases inhibitory activity against ABCG2, whereas III) the exchange of a carbonyl group with a methylene group at position C-5 increases it.

The importance of the pyrrolo[3,4-c] ring for inhibitory activities against PKC or cyclin-dependent kinases (CDK) has been shown for indolocarbazoles by co-crystallisation studies.[51] Indolo[2,3-a]pyrrolo[3,4-c]carbazoles, such as staurosporine or arcyriaflavin A and derivatives, are able to bind to the ATP binding site of various kinases by creating hydrogen bonds between amino acids residues and the pyrrolo[3,4-c] ring, whereby the binding of ATP to the kinase is prevented. Interestingly, **7** and **8** did not appreciably affect the ATPase activity of ABCG2.[38] The modification of the pyrrolo[3,4-c] ring of **8** showed different results regarding the PKC and D1-CDK4 inhibitory activity. The exchange of a carbonyl group with a methylene or hydroxy group decreased the inhibitory activity against the D1-CDK4 enzyme complex.[52] However, a methylene or hydroxy group increased the inhibitory activity against PKC and enhanced the antiproliferative activities against murine b16 melanoma cells.[53] The modification of the C-3 and/or C-9 position by halogenation has been shown to reduce various activities of **8**. Extensive structure-activity relationship studies revealed that a bromine substituent at C-3 position of **8** shows lower CDK4 inhibition,[54] whereas the addition of chlorine at positions C-3 and C-9 decreases the inhibitory activity against PKC.[44] Although the addition of two chlorine substituents reduced the PKC inhibitory activity, the activity against the human cytomegalovirus was maintained.[44] Our results are thus in agreement with the literature regarding the importance of the pyrrolo[3,4-c] ring as well as the absence of two chlorine substituents for inhibitory activity against PKC and CDK, and strengthen the key role of these structural features for the inhibition of the ABCG2 transporter.

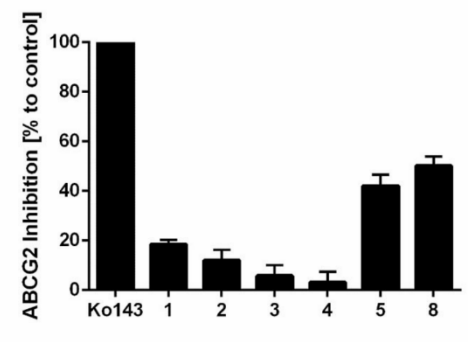


Figure 3. Inhibitory activity of tjipanazoles **1–5** and arcyriaflavin A (**8**) against ABCG2 at 10 μM, $n = 4$. Ko143 was used as positive control, indicating complete inhibition.

Conclusion

In conclusion, three tjipanazoles new to nature (**3**–**5**), were isolated along with two previously described tjipanazoles (**1**, **2**) from the cyanobacterium *F. ambigua* after a chemical screening using the software tool HaloSeeker. The putative BGC of the tjipanazoles has been refined. We substantiated the putative tjipanazole biosynthesis pathway based on new insights into the BGC as well as the structural diversity of the tjipanazoles produced by this strain. Tjipanazole M (**5**) showed similar inhibitory activity against ABCG2 as arcyriaflavin A. Structure-activity relationships point to the importance of the pyrrolo[3,4-c] ring as well as the chlorination as key features for the inhibition of ABCG2.

Experimental Section

General experimental procedures: HRMS data were acquired using a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific, Bremen, Germany) equipped with a heated ESI interface coupled to an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Bremen, Germany). HaloSeeker 1.0 was applied to HRMS data of extracts and fractions to detect HNPs.[34] Flash chromatography was performed on a GX-271 Liquid Handler system equipped with a 322 series pump and a 171 series diode array detector (Gilson, Middleton, USA). Semi-preparative HPLC was conducted on an UltiMate 3000 HPLC system (Thermo Fisher Scientific, Bremen, Germany). NMR spectra were either recorded at 600 MHz/150 MHz ($^{1}H/^{13}C$) on a Varian/Agilent V NMRS spectrometer, or at 400 MHz (^{1}H frequency) on an Agilent DD2 spectrometer. NMR spectra were analysed with ACD/Structure Elucidator Suite (2018.2, Toronto, Canada). Arcyriaflavin A (purity ≥ 95 %) was purchased from Cayman Chemical Company. Ko143 (purity ≥ 99 %) was purchased from Tocris Bioscience (Bristol, United Kingdom). All other chemicals were purchased from Sigma-Aldrich (Taufkirchen, Germany). Stock solutions of the compounds at a concentration of 10 mM were prepared in DMSO and stored at −18 °C for further usage. The prepared Krebs-HEPES buffer (KHB) contained 118.6 mM NaCl,

4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM CaCl, 11.7 mM D-glucose monohydrate, and 10.0 mM HEPES in doubly distilled water. The pH of the buffer has been adjusted to 7.41 at 37 °C using NaOH solution and then sterilised using a 0.2 μM pore membrane filter (Whatman, Maidstone, UK).

Cyanobacteria material: *Fischerella ambigua* (Näg.) Gomont 108b was classified as *Fischerella* based on its morphology and is deposited in the culture collection of the Cyano Biotech GmbH, Germany, under the accession number CBT 45. The strain was cultivated in BG-11 medium[55] at 28 °C, illuminated continuously by Sylvania GROLUX fluorescent lamps (50–200 μmol photons $m^{-2} s^{-1}$), and aerated with 0.5–5% CO_2 in sterile filtrated air in 20 L polycarbonate carboys. To minimise cell death and lysis, the cultures were harvested weekly and diluted with fresh medium (semi-continuous cultivation to avoid entering into stationary phase). Sufficient amounts of cyanobacteria biomass for further processing were obtained after a cultivation duration of about 10 weeks. After separation of the biomass from the medium by centrifugation, the biomass was lyophilised.

Extraction and isolation of compounds 1–5: 38 g of lyophilised dry biomass were suspended in 500 mL of 50% methanol in water (*v/v*), treated with an ultrasonication rod (Bandelin, Berlin, Germany), and extracted on a shaker for 30 min at room temperature. After centrifugation (20 min, 10800), the biomass was extracted using 500 mL 80% methanol (*v/v*). The solutions were combined and dried *in vacuo*, yielding 3.2 g of biomass extract. For preparative isolation of the active compounds, 1.6 g of the extract was dissolved in MeOH and fractionated by flash chromatography using a C_{18} flash cartridge (43 g sorbent, RS 40 C_{18} ec, Chromabond Flash, MACHEREY-NAGEL, Düren, Germany) and a binary gradient from 5–100% MeOH in water (0.1% formic acid each) as the mobile phase at 20 mL/min in 35 min, and a final elution step with 100% MeOH for 10 min to afford 24 fractions. Fractions 18–20 (t_R = 32–38 min) were combined, since most HNPs could be found in these fractions. The combined fraction (790 mg) was further purified by semi-preparative HPLC using a C_{18} column (250 x 100 mm, 5 μm, 100 Å, Luna, Phenomenex, Aschaffenburg, Germany) and a step gradient of ACN in water (0.1% formic acid each) at 4.7 mL/min, starting with 57% ACN for 16 min, stepping to 70% for 6 min, increasing to 100% within 5 min and a final step with 100% ACN for 10 min. Several rounds of separation afforded tjipanazole K (**3**; t_R = 10.8 min, 0.9 mg), tjipanazole M (**5**; t_R = 13.3 min, 1.0 mg), tjipanazole L (**4**; t_R = 19.9 min, 8.0 mg), tjipanazole I (**2**; t_R = 25.9 min, 0.8 mg) and tjipanazole D (**1**; t_R = 28.6 min, 14.8 mg).

Tjipanazole D (**1**): brown, amorphous powder; NMR spectra: see Figure S3; HRMS (ESI): *m/z* calcd for $C_{18}H_{11}Cl_2N_2$-H^-: 323.0148 [*M*-H]$^-$; found: 323.0146.

Tjipanazole I (**2**): brown, amorphous powder; NMR spectra: see Figures S4 and S5; HRMS (ESI): *m/z* calcd for $C_{18}H_{11}ClN_2$-H^-: 289.0538 [*M* - H]$^-$; found: 289.0538.

Tjipanazole K (**3**): yellow crystals; NMR (^{1}H 600 MHz, ^{13}C 150 MHz, [D_6]DMSO): δ_H 4.98 (s, 1H; H-5), 7.40 (dd, *J* = 8.4, 2.2 Hz, 1H; H-10), 7.46 (dd, *J* = 8.5, 1.9 Hz, 1H; H-2), 7.63 (d, *J* = 8.4 Hz, 1H; H-11), 7.68 (d, *J* = 8.6 Hz, 1H; H-1), 8.05 (d, *J* = 1.8 Hz, 1H; H-4), 9.23 (d, *J* = 2.0 Hz, 1H; H-8), 13.95 (br s, 1NH), 14.22 (br s, 1NH,); δ_C 45.8 (C-5), 113.3 (C-11), 113.9 (C-1), 120.9 (C-4), 124.5 (C-8), 125.1 (C-10), 125.3 ppm (C-2); NMR spectra: see Figures S6–S8; HRMS (ESI): *m/z* calcd for $C_{20}H_{11}Cl_2N_3O$-H^-: 378.0206 [*M*-H]$^-$; found: 378.0207.

Tjipanazole L (**4**): yellow crystals; NMR (^{1}H 400 MHz, ^{13}C 100 MHz, [D_6]DMSO): δ_H 7.57 (dd, *J* = 8.8, 2.2 Hz, 2H; H-2, H-10), 7.81 (d, *J* = 8.6 Hz, 2H; H-1, H-11), 8.96 (d, *J* = 2.2 Hz, 2H; H-4, H-8), 11.1 (br s, 1NH), 12.98 ppm (br s, 2NH); δ_C 113.7 (C-1, C-11), 114.6 (C-4b, C-7b), 120.2 (C-4c, C-7a), 122.5 (C-4a, C-7c), 123.1 (C-4, C-8), 124.3 (C-3, C-9), 126.5 (C-2, C-10), 129.6 (C-12a, C-12b), 138.7 (C-11a, 13-a), 171.1 ppm (C-5, C-7); NMR spectra: see Figures S9–S12; HRMS (ESI): *m/z* calcd for $C_{20}H_9Cl_2N_3O_2$-H^-: 391.9999 [*M*-H]$^-$; found: 391.9997.

Tjipanazole M (**5**): yellow crystals; NMR (^{1}H 600 MHz, ^{13}C 150 MHz, [D_6]DMSO): δ_H 7.32 (t, *J* = 7.5 Hz, 1H; H-3), 7.53 (m, 2H; H-2, H-10), 7.69 (m, 2H; H-1, H-11), 8.97 (m, 2H, H-4, H-8), 10.96 (s, 1NH), 14.48 (br s, 1NH), 14.74 (br s, 1NH); δ_C 111.7 (C-1), 113.1 (C-11), 119.6 (C-3), 122.9 (C-8), 123.9 (C-4), 126.0 (C-10), 126.1 ppm (C-2); NMR spectra: see Figures S13–S15; HRMS (ESI): *m/z* calcd for $C_{20}H_{10}ClN_3O_2$-H^-: 358.0389 [*M*-H]$^-$; found: 358.0390.

DNA isolation and sequencing: Axenic *F. ambigua* cells were inoculated in 100 mL BG-11 medium and grown at 25 °C with shaking at 120 rpm under constant illumination. Cells were harvested after 2–3 weeks of growth, washed three times with sterile water and resuspended in 20 mL SET buffer (75 mM NaCl, 25 mM EDTA, 10 mM Tris-HCl). The filaments were mechanically broken to separate the cells using a homogeniser. SDS (0.5%), proteinase K (500 μg/mL) and lysozyme (2.5 mg/mL) were added and the suspension was incubated at 55 °C for 2 h. Subsequently, one volume of phenol/chloroform/isoamyl alcohol (25:24:1) was added for extraction. This step was repeated until no precipitated proteins could be seen between the aqueous and the organic phase. The resulting supernatant was purified using genomic tips, which were supplied with the Blood & Cell Culture DNA Mini Kit (Qiagen, Hilden, Germany) based on the manufacturer's instruction manual. The resulting DNA was submitted to 454 sequencing applying the Roche GS FLX Titanium sequencer (GATC, Konstanz, Germany). The sequence of the putative tjipanazole BGC has been deposited in GenBank (MT078730).

Quantification by evaporative light scattering detection: The concentrations of test compound solutions for the ABCG2 assay were quantified using HPLC coupled with an evaporative light scattering detector (ELSD; Sedex 85, Sedere).[56] Tjipanazole L (**4**) was used as standard substance to establish the calibration curve (injection of 0.5 to 6 μL of a 150 ng/μL solution in DMSO). Solutions were injected in triplicate on a Kinetex C_{18} column (100 × 3.0 mm, 2.6 μm, 100 Å, Phenomenex) and eluted with a step gradient of ACN in water (0.1% formic acid each) at 0.85 mL/min, starting with 40% ACN for 1 min and stepping to 60% ACN for 6 min. ELSD response areas were averaged and log(ELSD response area) was plotted against log(amount) to generate a linear calibration curve. Solutions of the compounds in DMSO were injected in triplicate under identical conditions. ELSD response areas were averaged, and the corresponding compound concentration was calculated using the tjipanazole L (**4**) calibration curve.

Cell culture: MDCK II BCRP cell line was a kind gift from Dr. A. Schinkel (The Netherlands Cancer Institute, Amsterdam, Netherlands). Cell culture was performed in Dulbecco's modified Eagle's medium (DMEM) containing 10% foetal calf serum (FCS), 2 mM L-glutamine, 50 μg/mL streptomycin and 50 U/mL penicillin G. MDCK II BCRP cells were incubated at 37 °C under humidified atmosphere containing 5% CO_2. At a confluence of 80–90%, sub-culturing was performed using 0.05% trypsin and 0.02% EDTA for detaching the cells from the inner surface of the culture flask. Then cells were resuspended with the fresh medium into a 50 ml falcon. A cell pellet was obtained by centrifugation (4 °C, 4 min, 266 × g) and the supernatant was removed by aspiration of the liquid. Then cells were again resuspended in fresh medium. The amount of cells was determined using a CASY1 model TT cell counter equipped with a 150 μm capillary (Schaerfe System GmbH, Reutlingen, Germany). Before using the cells in Hoechst 33342 accumulation assay, they were washed three times with KHB to remove residual medium.

Hoechst 33342 accumulation assay: The inhibitory effect of the compounds was investigated in the Hoechst 33342 accumulation assay using ABCG2 transfected MDCK II BCRP cells as described in the literature with minor modifications.[57] Bisbenzimidazole derivative, Hoechst 33342 is a fluorescent compound and a substrate of ABCG2 which has a stronger fluorescence after binding into adenine- and thymine-rich minor groves of double-stranded DNA or being embedded in a lipophilic environment like the cell membrane. For this aim, a stock solution of the compounds was prepared at a final concentration of 10 mM in DMSO. From this solution, 11 different concentrations were prepared using sterile KHB and a small amount of methanol. The final concentration of the DMSO and methanol in the presence of cells was always below 0.1 % and 5 %, respectively. Assays were performed on BMG POLARstar microplate reader (BMG Labtech, Offenburg, Germany) at 37 °C (excitation: 355 nm/emission: 460 nm) using 96-well black plates (Greiner, Frickenhausen, Germany). Black plates were favourable as they provided much lower background fluorescence than transparent plates when irradiated in the UV. The cells were prepared as described above. 160 μL cell suspension in KHB at a cell density of approximately 30 000 cells per well was added to each well together with 20 μL of the different concentrations of the test compounds. The plate was then incubated for 30 min at 37 °C and 5 % CO_2 followed by quick addition of 20 μL of a 10 μM Hoechst 33342 solution (protected from light) to each well. Fluorescence intensity was measured immediately for the next 120 min at constant time intervals of 1 min. The average fluorescence between 100 and 109 min in the steady state was calculated and plotted against the logarithm of the compound concentration. Dose-response curves were generated using non-linear regression analysis, the four-parameter logistic equation with variable slope compared to the three-parameter logistic equation. The statistically preferred model was selected for calculating IC_{50} values (GraphPad Prism, version 6.0, San Diego, USA). For compounds reaching the maximal effect of Ko143 up to 10 μM, IC_{50} values were calculated by constraining the maximal response to that of Ko143. Percentage of the response of the compounds in comparison to the reference inhibitor (100 % inhibition), has been calculated by comparing the fluorescence intensity of the compounds at their highest concentration in comparison to Ko143.

Conflict of Interest

H.E. is CSO and co-owner of Cyano Biotech GmbH; the company does not have any financial interest in the research presented here. The authors declare no conflict of interest. The funding sponsors had no role in the design, writing or publishing strategy of the study, or in the collection, analysis or interpretation of the data.

Acknowledgements

This work was financially supported by the German Research Foundation (DFG; INST 271/388-1; T.H.J.N.). We thank Andrea Porzel (Institute for Plant Biochemistry, Halle, Germany) for recording the NMR spectra.

Keywords: biosynthesis · cancer resistance protein · *Fischerella* · natural products · tjipanazoles

[1] G. W. Gribble, *Environ. Chem.* **2015**, *12*, 39.
[2] G. W. Gribble, *Mar. Drugs* **2015**, *13*, 4044–4136.
[3] D. P. Levine, *Clin. Infect. Dis.* **2006**, *42 Suppl 1*, 5–12.
[4] P. J. Ambrose, *Clin. Pharmacokinet.* **1984**, *9*, 222–238.
[5] J. A. Bush, B. H. Long, J. J. Catino, W. T. Bradner, K. Tomita, *J. Antibiot.* **1987**, *40*, 668–678.
[6] G. Trimurtulu, I. Ohtani, G. M. L. Patterson, R. E. Moore, T. H. Corbett, F. A. Valeriote, L. Demchik, *J. Am. Chem. Soc.* **1994**, *116*, 4729–4737.
[7] K. Naumann, *Pest Manage. Sci.* **2000**, *56*, 3–21.
[8] C. M. Harris, R. Kannan, H. Kopecka, T. M. Harris, *J. Am. Chem. Soc.* **1985**, *107*, 6652–6658.
[9] R. Wilcken, M. O. Zimmermann, A. Lange, A. C. Joerger, F. M. Boeckler, *J. Med. Chem.* **2013**, *56*, 1363–1388.
[10] M. T. Cabrita, C. Vale, A. P. Rauter, *Mar. Drugs* **2010**, *8*, 2301–2317.
[11] a) V. Agarwal, A. A. El Gamal, K. Yamanaka, D. Poth, R. D. Kersten, M. Schorn, E. E. Allen, B. S. Moore, *Nat. Chem. Biol.* **2014**, *10*, 640–647; b) N. Kasanah, T. Triyanto, *Biomolecules* **2019**, *9*, 225.
[12] a) R. E. Moore, *Acc. Chem. Res.* **1977**, *10*, 40–47; b) Y. Takahashi, M. Suzuki, T. Abe, M. Masuda, *Phytochemistry* **1999**, *50*, 799–803; c) N.-Y. Ji, X.-M. Li, B.-G. Wang, *Helv. Chim. Acta* **2010**, *93*, 2281–2286; d) M. Liu, P. E. Hansen, X. Lin, *Mar. Drugs* **2011**, *9*, 1273–1292; e) V. H. Woolner, C. M. Jones, J. J. Field, N. H. Fadzilah, A. B. Munkacsi, J. H. Miller, R. A. Keyzers, P. T. Northcote, *J. Nat. Prod.* **2016**, *79*, 463–469; f) A. Jesus, M. Correia-da-Silva, C. Afonso, M. Pinto, H. Cidade, *Mar. Drugs* **2019**, *17*.
[13] a) N. K. Utkina, S. A. Fedoreyev, S. G. Ilyin, M. Y. Antipin, *Russ. Chem. Bull.* **1998**, *47*, 2292–2294; b) N. B. Pham, M. S. Butler, J. N. Hooper, R. W. Moni, R. J. Quinn, *J. Nat. Prod.* **1999**, *62*, 1439–1442; c) T. Kusama, N. Tanaka, K. Sakai, T. Gonoi, J. Fromont, Y. Kashiwada, J. i Kobayashi, *Org. Lett.* **2014**, *16*, 3916–3918; d) J.-H. Sheu, P.-J. Sung, J.-H. Su, H.-Y. Liu, C.-Y. Duh, M. Y. Chiang, *Tetrahedron* **1999**, *55*, 14555–14564; e) A. Qureshi, D. J. Faulkner, *Nat. Prod. Lett.* **1999**, *13*, 59–62; f) A. Rudi, T. Evan, M. Aknin, Y. Kashman, *J. Nat. Prod.* **2000**, *63*, 832–833; g) D. Smitha, M. M. K. Kumar, H. Ramana, D. V. Rao, *Nat. Prod. Res.* **2014**, *28*, 12–17.
[14] a) J. L. Varns, *Am. Potato J.* **1982**, *59*, 593–604; b) J. Gan, S. R. Yates, H. D. Ohr, J. J. Sims, *Geophys. Res. Lett.* **1998**, *25*, 3595–3598; c) Y. Zhu, L. Yang, Z. J. Jia, *J. Nat. Prod.* **1999**, *62*, 1479–1483; d) S. R. Jensen, C. H. Gotfredsen, U. S. Harput, I. Saracoglu, *J. Nat. Prod.* **2010**, *73*, 1593–1596; e) J. Wang, N. Han, Y. Wang, Y. Wang, Z. Liu, Y. Wang, J. Yin, *Nat. Prod. Res.* **2014**, *28*, 1306–1309.
[15] a) S. Nakanishi, K. Ando, I. Kawamoto, T. Yasuzawa, H. Sano, H. Kase, *J. Antibiot.* **1989**, *42*, 1775–1783; b) P. J. Silk, C. Aubry, G. C. Lonergan, J. B. Macaulay, *Chemosphere* **2001**, *44*, 1603–1616; c) A. E. de Jesus, P. S. Steyn, F. R. van Heerden, R. Vleggaar, P. L. Wessels, W. E. Hull, *J. Chem. Soc. Perkin Trans. 1* **1983**, 1847–1856; d) J. A. Field, F. J. M. Verhagen, E. de Jong, *Trends Biotechnol.* **1995**, *13*, 451–456; e) M. Ishibashi, A. Shintani, K. Toume, Y. Yamamoto, *Heterocycles* **2010**, *82*, 839; f) Y.-C. Chang, C.-K. Lu, Y.-R. Chiang, G.-J. Wang, Y.-M. Ju, Y.-H. Kuo, T.-H. Lee, *J. Nat. Prod.* **2014**, *77*, 751–757.
[16] a) J. Breinholt, H. Gürtler, A. Kjær, S. E. Nielsen, C. E. Olsen, K. Ranta, T. Rojo, *Acta Chem. Scand.* **1998**, *52*, 1040–1044; b) D. S. Fukuda, J. S. Mynderse, P. J. Baker, D. M. Berry, L. D. Boeck, R. C. Yao, F. P. Mertz, W. M. Nakatsukasa, J. Mabe, J. Ott, *J. Antibiot.* **1990**, *43*, 623–633; c) D. C. Hopp, J. Rhea, D. Jacobsen, K. Romari, C. Smith, J. Rabenstein, M. Irigoyen, M. Clarke, L. Francis, M. Luche, G. J. Carr, U. Mocek, *J. Nat. Prod.* **2009**, *72*, 276–279; d) R. Jansen, S. Sood, V. Huch, B. Kunze, M. Stadler, R. Müller, *J. Nat. Prod.* **2014**, *77*, 320–326.
[17] a) D. Li, O. Sihamala, S. Bhulaidok, L. Shen, *Acta Aliment.* **2009**, *38*, 493–501; b) M. A. K. Khalil, R. A. Rasmussen, J. R. J. French, J. A. Holt, *J. Geophys. Res.* **1990**, *95*, 3619; c) R. S. Berger, *J. Med. Entomol.* **1983**, *20*, 103.
[18] a) T. F. Spande, H. M. Garraffo, M. W. Edwards, H. J. C. Yeh, L. Pannell, J. W. Daly, *J. Am. Chem. Soc.* **1992**, *114*, 3475–3478; b) R. W. Fitch, T. F. Spande, H. M. Garraffo, H. J. C. Yeh, J. W. Daly, *J. Nat. Prod.* **2010**, *73*, 331–337.
[19] a) J. Demay, C. Bernard, A. Reinhardt, B. Marie, *Mar. Drugs* **2019**, *17*; b) T. H. Niedermeyer, *Planta Med.* **2015**, *81*, 1309–1325.
[20] M. Murakami, K. Ishida, T. Okino, Y. Okita, H. Matsuda, K. Yamaguchi, *Tetrahedron Lett.* **1995**, *36*, 2785–2788.
[21] a) U. Matern, L. Oberer, R. A. Falchetto, M. Erhard, W. A. König, M. Herdman, J. Weckesser, *Phytochemistry* **2001**, *58*, 1087–1095; b) S. Gesner-Apter, S. Carmeli, *J. Nat. Prod.* **2009**, *72*, 1429–1436.
[22] T. Golakoti, J. Ogino, C. E. Heltzel, T. Le Husebo, C. M. Jensen, L. K. Larsen, G. M. L. Patterson, R. E. Moore, S. L. Mooberry, T. H. Corbett, F. A. Valeriote, *J. Am. Chem. Soc.* **1995**, *117*, 12030–12049.
[23] K. Ishida, H. Matsuda, M. Murakami, *Tetrahedron* **1998**, *54*, 13475–13484.

[24] H. Luesch, W. Y. Yoshida, R. E. Moore, V. J. Paul, S. L. Mooberry, *J. Nat. Prod.* **2000**, *63*, 611–615.
[25] T. A. Smitka, R. Bonjouklian, L. Doolin, N. D. Jones, J. B. Deeter, W. Y. Yoshida, M. R. Prinsep, R. E. Moore, G. M. L. Patterson, *J. Org. Chem.* **1992**, *57*, 857–861.
[26] R. E. Moore, C. Cheuk, G. M. L. Patterson, *J. Am. Chem. Soc.* **1984**, *106*, 6456–6457.
[27] K. Stratmann, R. E. Moore, R. Bonjouklian, J. B. Deeter, G. M. L. Patterson, S. Shaffer, C. D. Smith, T. A. Smitka, *J. Am. Chem. Soc.* **1994**, *116*, 9935–9942.
[28] A. Park, R. E. Moore, G. M. L. Patterson, *Tetrahedron Lett.* **1992**, *33*, 3257–3260.
[29] B. S. Falch, G. M. Koenig, A. D. Wright, O. Sticher, H. Ruegger, G. Bernardinelli, *J. Org. Chem.* **1993**, *58*, 6570–6575.
[30] P. N. Leão, H. Nakamura, M. Costa, A. R. Pereira, R. Martins, V. Vasconcelos, W. H. Gerwick, E. P. Balskus, *Angew. Chem. Int. Ed.* **2015**, *54*, 11063–11067; *Angew. Chem.* **2015**, *127*, 11215–11219.
[31] H. T. N. Bui, R. Jansen, H. T. L. Pham, S. Mundt, *J. Nat. Prod.* **2007**, *70*, 499–503.
[32] J. H. Cardellina, F. J. Marner, R. E. Moore, *J. Am. Chem. Soc.* **1979**, *101*, 240–242.
[33] R. Bonjouklian, T. A. Smitka, L. E. Doolin, R. M. Molloy, M. Debono, S. A. Shaffer, R. E. Moore, J. B. Stewart, G. M. L. Patterson, *Tetrahedron* **1991**, *47*, 7739–7750.
[34] A. Léon, R. Cariou, S. Hutinet, J. Hurel, Y. Guitton, C. Tixier, C. Munschy, J.-P. Antignac, G. Dervilly-Pinel, B. Le Bizec, *Anal. Chem.* **2019**, *91*, 3500–3507.
[35] a) *Dictionary of Natural Products 28.1*, http://dnp.chemnetbase.com/faces/chemical/ChemicalSearch.xhtml; b) S. Mo, A. Krunic, G. Chlipala, J. Orjala, *J. Nat. Prod.* **2009**, *72*, 894–899; c) T. A. Smitka, R. Bonjouklian, L. Doolin, N. D. Jones, J. B. Deeter, W. Y. Yoshida, M. R. Prinsep, R. E. Moore, G. M. L. Patterson, *J. Org. Chem.* **1992**, *57*, 857–861; d) H. Kim, A. Krunic, D. Lantvit, Q. Shen, D. J. Kroll, S. M. Swanson, J. Orjala, *Tetrahedron* **2012**, *68*, 3205–3209; e) A. Park, R. E. Moore, G. M. L. Patterson, *Tetrahedron Lett.* **1992**, *33*, 3257–3260; f) S. Mo, A. Krunic, B. D. Santarsiero, S. G. Franzblau, J. Orjala, *Phytochemistry* 2010, 71, 2116–2123; g) J. I. Jimenez, U. Huber, R. E. Moore, G. M. Patterson, *J. Nat. Prod.* **1999**, *62*, 569–572; h) R. E. Schwartz, C. F. Hirsch, J. P. Springer, D. J. Pettibone, D. L. Zink, *J. Org. Chem.* **1987**, *52*, 3704–3706.
[36] A. D. Wright, O. Papendorf, G. M. Konig, *J. Nat. Prod.* **2005**, *68*, 459–461.
[37] M. El Omari, PhD Thesis, University of Bonn, Germany, 2011.
[38] R. W. Robey, S. Shukla, K. Steadman, T. Obrzut, E. M. Finley, S. V. Ambudkar, S. E. Bates, *Mol. Cancer Ther.* **2007**, *6*, 1877–1885.
[39] a) M. L. H. Vlaming, J. S. Lagas, A. H. Schinkel, *Adv. Drug Delivery Rev.* **2009**, *61*, 14–25; b) W. Mo, J.-T. Zhang, *Int. J. Biochem. Mol. Biol.* **2011**, *3*, 1–27.
[40] a) A. Ahmed-Belkacem, A. Pozza, S. Macalou, J. M. Pérez-Victoria, A. Boumendjel, A. Di Pietro, *Anti-Cancer Drugs* **2006**, *17*, 239–243; b) F. Lecerf-Schmidt, B. Peres, G. Valdameri, C. Gauthier, E. Winter, L. Payen, A. Di Pietro, A. Boumendjel, *Future Med. Chem.* **2013**, *5*, 1037–1045; c) M. H. Hasanabady, F. Kalalinia, *Journal of biosciences* **2016**, *41*, 313–324.
[41] A. D. Wright, O. Papendorf, G. M. König, A. Oberemm, *Chemosphere* **2006**, *65*, 604–608.
[42] T. Yasuzawa, T. Iida, M. Yoshida, N. Hirayama, M. Takahashi, K. Shirahata, H. Sano, *J. Antibiot.* **1986**, *39*, 1072–1078.
[43] a) J. Kleinschroth, H. Barth, J. Hartenstein, C. Schaechtele, C. Rudolph, H. Osswald, DE3835842 (A1), 1990; b) J. Kleinschroth, J. Hartenstein, C. Schaechtele, C. Rudolph, D. Marme, S. Paetzold, DE4217963 (A1), 1993.
[44] M. J. Slater, S. Cockerill, R. Baxter, R. W. Bonser, K. Gohil, C. Gowrie, J. E. Robinson, E. Littler, N. Parry, R. Randall, W. Snowden, *Bioorg. Med. Chem.* **1999**, *7*, 1067–1074.
[45] J. Bergman, B. Pelcman, *J. Org. Chem.* **1989**, *54*, 824–828.
[46] C. Sánchez, L. Zhu, A. F. Braña, A. P. Salas, J. Rohr, C. Méndez, J. A. Salas, *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 461–466.
[47] a) H. Onaka, S. -i Taniguchi, Y. Igarashi, T. Furumai, *J. Antibiot.* **2002**, *55*, 1063–1071; b) A. P. Salas, L. Zhu, C. Sánchez, A. F. Braña, J. Rohr, C. Méndez, J. A. Salas, *Mol. Microbiol.* **2005**, *58*, 17–27.
[48] M. Makino, H. Sugimoto, Y. Shiro, S. Asamizu, H. Onaka, S. Nagano, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 11591–11596.
[49] B. S. Falch, G. M. König, A. D. Wright, O. Sticher, C. K. Angerhofer, J. M. Pezzuto, H. Bachmann, *Planta Med.* **1995**, *61*, 321–328.
[50] R. W. Robey, K. Steadman, O. Polgar, K. Morisaki, M. Blayney, P. Mistry, S. E. Bates, *Cancer Res.* **2004**, *64*, 1242–1246.
[51] a) C. Sanchez-Martinez, C. Shih, G. Zhu, T. Li, H. B. Brooks, B. K. R. Patel, R. M. Schultz, T. B. DeHahn, C. D. Spencer, S. A. Watkins, C. A. Ogg, E. Considine, J. A. Dempsey, F. Zhang, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3841–3846; b) O. A. B. S. M. Gani, R. A. Engh, *Nat. Prod. Rep.* **2010**, *27*, 489–498.
[52] C. Sanchez-Martinez, C. Shih, M. M. Faul, G. Zhu, M. Paal, C. Somoza, T. Li, C. A. Kumrich, L. L. Winneroski, Z. Xun, H. B. Brooks, B. K. R. Patel, R. M. Schultz, T. B. DeHahn, C. D. Spencer, S. A. Watkins, E. Considine, J. A. Dempsey, C. A. Ogg, R. M. Campbell, B. A. Anderson, J. Wagner, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3835–3839.
[53] E. R. Pereira, L. Belin, M. Sancelme, M. Prudhomme, M. Ollier, M. Rapp, D. Sevère, J. F. Riou, D. Fabbro, T. Meyer, *J. Med. Chem.* 1996, 39, 4471–4477.
[54] G. Zhu, S. E. Conner, X. Zhou, C. Shih, T. Li, B. D. Anderson, H. B. Brooks, R. M. Campbell, E. Considine, J. A. Dempsey, M. M. Faul, C. Ogg, B. Patel, R. M. Schultz, C. D. Spencer, B. Teicher, S. A. Watkins, *J. Med. Chem.* **2003**, *46*, 2027–2030.
[55] R. A. Andersen, *Algal Culturing Techniques*, Elsevier Academic Press, Burlington, MA, 2005.
[56] N. Adnani, C. R. Michel, T. S. Bugni, *J. Nat. Prod.* **2012**, *75*, 802–806.
[57] S. C. Köhler, M. Wiese, *J. Med. Chem.* **2015**, *58*, 3910–3921.

Manuscript received: January 17, 2020
Revised manuscript received: February 21, 2020
Accepted manuscript online: March 17, 2020
Version of record online: April 16, 2020

3.2 Publication 2: "Hapalindoles from the Cyanobacterium *Hapalosiphon* sp. Inhibit T Cell Proliferation"

Tomasz Chilczuk, Carmen Steinborn, Steffen Breinlinger, Amy M. Zimmermann-Klemd, Roman Huber, Heike Enke, Dan Enke, Timo H. J. Niedermeyer, Carsten Gründemann
Planta Medica 2020, *86*, 96–103, DOI: 10.1055/a-1045-5178
The Supporting Information is available at
https://www.thieme-connect.com/products/ejournals/abstract/10.1055/a-1045-5178.

Immunomodulatory drugs are currently sought after for their ability to stimulate or suppress the immune system and help the body to fight cancer or autoimmune diseases. Therefore, this study aimed to identify and characterize new immunomodulating natural products from cyanobacteria. A cyanobacteria extract collection was screened for immunomodulating compounds resulting in the identification of 35 active cyanobacteria extracts. Bioactivity-guided fractionation of a *Hapalosiphon* sp. CBT1235 extract showed that the major compounds of the extract caused the attenuation of T cell proliferation. Thus, five compounds responsible for the observed bioactivity were isolated by semi-preparative HPLC. All compounds belong to the chemical family known as hapalindoles. Their structures were elucidated based on 1D, 2D NMR and HRMS data. NOESY experiments and specific rotation values were used to determine the absolute configuration of the hapalindoles. The previously already isolated compounds, hapalindole A, D, and M, were dereplicated. The two remaining isolated compounds, hapalindole A-formamide and hapalindole J-formamide, were not yet described as natural products.
Detailed characterization of the immunomodulating activities of the pure compounds showed that all hapalindoles suppressed T cell proliferation, with hapalindole A exhibiting the highest antiproliferative activity. Except for hapalindole J-formamide, all hapalindoles showed apoptotic effects at high concentrations. Although, hapalindole A induced T cell apoptosis, attenuation of T cell proliferation was measurable at up to 10-fold lower concentrations, making this compound a potential candidate for the development of anti-inflammatory drugs. Moreover, the conducted structure-activity relationship study highlights the importance of the isonitrile functional group for the antiproliferative bioactivity of the hapalindoles.

Hapalindoles from the Cyanobacterium *Hapalosiphon* sp. Inhibit T Cell Proliferation

Authors

Tomasz Chilczuk[1§], Carmen Steinborn[2§], Steffen Breinlinger[1§], Amy Marisa Zimmermann-Klemd[2], Roman Huber[2], Heike Enke[3], Dan Enke[3], Timo Horst Johannes Niedermeyer[1Δ]*, Carsten Gründemann[2Δ]*

Affiliations

[1] Department of Pharmaceutical Biology/Pharmacognosy, Institute of Pharmacy, University of Halle-Wittenberg, Halle, Germany

[2] Center for Complementary Medicine, Institute for Infection Prevention and Hospital Epidemiology, Faculty of Medicine, University of Freiburg, Freiburg, Germany

[3] Cyano Biotech GmbH, Berlin, Germany

[§, Δ] These authors contributed equally to this work

Keywords

Cyanobacteria, *Hapalosiphon*, Hapalindoles, Immune Modulation, Lymphocytes

Abbreviations

CFSE - carboxyfluorescein diacetate succinimidyl ester; CPT – camptothecin; CsA – cyclosporine A; PBMC – peripheral blood mononuclear cells

***Corresponding authors**

PD Dr. Carsten Gründemann, Center for Complementary Medicine, Institute for Infection Prevention and Hospital Epidemiology, Faculty of Medicine, Breisacherstrasse 115B, 79106 Freiburg, Germany; carsten.gruendemann@uniklinik-freiburg.de; Phone: +49 / (0)761 / 270-83170; Fax: +49 / (0)761 / 270-83230

Prof. Dr. Timo H. J. Niedermeyer, Department of Pharmaceutical Biology/Pharmacognosy, Institute of Pharmacy, Hoher Weg 8, 06120 Halle (Saale), Germany; timo.niedermeyer@pharmazie.uni-halle.de; Phone: +49 / (0)345 / 55-25765; Fax: +49 / (0)345 / 55-27407

Abstract

Novel immunomodulating agents are currently sought after for the treatment of autoimmune diseases and cancers. In this context, a screening campaign of a collection of 575 cyanobacteria extracts for immunomodulatory effects has been conducted.. The screening resulted in several active extracts. Here we report the results of subsequent studies on an extract from the cyanobacterium *Hapalosiphon* sp. CBT1235. We identified five hapalindoles as the compounds responsible for the observed immunomodulatory effect. These indole alkaloids are produced by several strains of the cyanobacterial family Hapalosiphonaceae. They are known for their anti-infective, cytotoxic, and other bioactivities. Modulation of the activity of human immune cells has not yet been described. The immunomodulatory activity of the hapalindoles was characterized *in vitro* using flow cytometry-based measurements of T cell proliferation after carboxyfluorescein diacetate succinimidyl ester staining, and apoptosis and necrosis induction after annexin V / propidium iodide staining. The most potent compound, hapalindole A, reduced T cell proliferation with an IC_{50} of 1.56 µM, while relevant levels of apoptosis were measurable only at ten-fold higher concentrations. Hapalindole A-formamide and hapalindole J-formamide, isolated for the first time from a natural source, had much lower activity than the non-formylated derivatives while, at the same time, being less selective for anti-proliferative over apoptotic effect.

Introduction

Cyanobacteria are an intriguing source for structurally diverse and biologically active natural products. Especially the genera *Microcystis*, *Nostoc*, and *Lyngbya* or *Moorea* are chemically well characterized [1–7]. Several strains of the family Hapalosiphonaceae produce indole alkaloids [8]. The major classes of these indole alkaloids include hapalindoles, ambiguines, fischerindoles, and welwitindolinones, of which more than 80 variants have been described in the literature [9–24]. They share several common motifs: an indole or oxindole core, a cyclohexane fused to that core, an isonitrile or isothiocyanate functional group, a chlorine substituent, or an additional ring [25]. The largest group of these alkaloids are the hapalindoles. So far 30 different hapalindoles have been reported [9, 10, 15, 18, 21, 26]. The first hapalindoles, hapalindoles A and B, were discovered in 1984 by Moore *et al*. from *Hapalosiphon fontinalis* [9]. Since then, diverse hapalindoles have been isolated from various strains of the genera *Hapalosiphon*, *Fischerella*, and *Westiellopsis* [9, 17, 21, 26].

The hapalindoles have a broad spectrum of biological activities, e.g. antimicrobial activity against bacteria [17, 19, 21, 27], fungi [19, 27] and algae [27]. Studies by Doan *et al*. suggested inhibition of RNA polymerase and consequently the disturbance of the protein biosynthesis as a possible mode of action for the antibacterial activity [27, 28]. Furthermore, cytotoxic activity against normal mammalian and numerous cancer cell lines has been reported for various hapalindoles [21, 27]. However, no mode of action for this activity has been described, yet. Finally, they have been shown to be toxic to insects [18, 29] and vertebrates [23]. The insecticidal activity could be explained by sodium channel modulating activity of the indole alkaloids [30]. Interestingly, inhibition of sodium channels did not lead to any cytotoxicity in neuroblastoma cell lines. Thus, compared to insects, a different mode of action must underlie the cytotoxicity on mammalian cells. Although various bioactivities can be attributed to hapalindoles, modulation of the activity of human immune cells has not yet been described. Immunomodulatory drugs are used as modifiers of the immune system to either enhance the immune response against infectious diseases, tumors and immunodeficiency, or to suppress the immune reaction in organ transplants or to treat autoimmune responses. Screening of a cyanobacteria extract collection (575 extracts) derived from strains from all cyanobacteria orders for inhibition of T cell proliferation resulted in 35 extracts with an activity at 1 µg/ml or lower. One of

the active extracts was derived from a *Hapalosiphon* sp. strain. Bioassay-guided fractionation of the extract led to the isolation of three known hapalindoles, hapalindole A (**1**) [9], D (**2**), and M (**3**) [10], as well as two formamide-bearing hapalindoles that are reported here for the first time from a natural source, namely hapalindole A-formamide (**4**) [31] and hapalindole J-formamide (**5**) [32]. Here, we report the isolation, structure elucidation, and the investigation of the immunomodulatory properties of the hapalindole derivatives isolated from *Hapalosiphon* sp. CBT1235.

Results and discussion

Our initial screening of a cyanobacteria extract collection for immunomodulating activity on human immune cells resulted in 35 extracts with an activity at a concentration of 1 µg/mL or lower. Nine out of the 11 most potent extracts were derived from cyanobacteria of the genus *Nostoc*. Dereplication of the natural products in these *Nostoc* strain extracts showed the presence of the well-known cytotoxic compound cryptophycin-1 in most of these strains [33–36], so they were excluded from further investigation. Chromatographic evaluation of the remaining extracts by HPLC-DAD/MS showed that few of them featured prominent peaks. In the HPLC-DAD chromatogram of one *Hapalosiphon* sp. extract, several prominent peaks were observed, thus this strain was selected for follow-up work. T cell proliferation was inhibited by this extract with an IC_{50} of 0.11 µg/mL (**Fig. 1A**). At the same time, a trend to an increased amount of apoptotic cells at an extract concentration of 10 µg/ml could be observed (statistically non-significant, **Fig. 1B**). No induction of necrosis has been observed (data not shown). Microfractionation of the extract and subsequent bioassays showed that indeed the major compounds observable in the chromatogram were responsible for the bioactivity and led to the significant retardation of T cell proliferation (**Fig. 1Sa-c** in the SI).

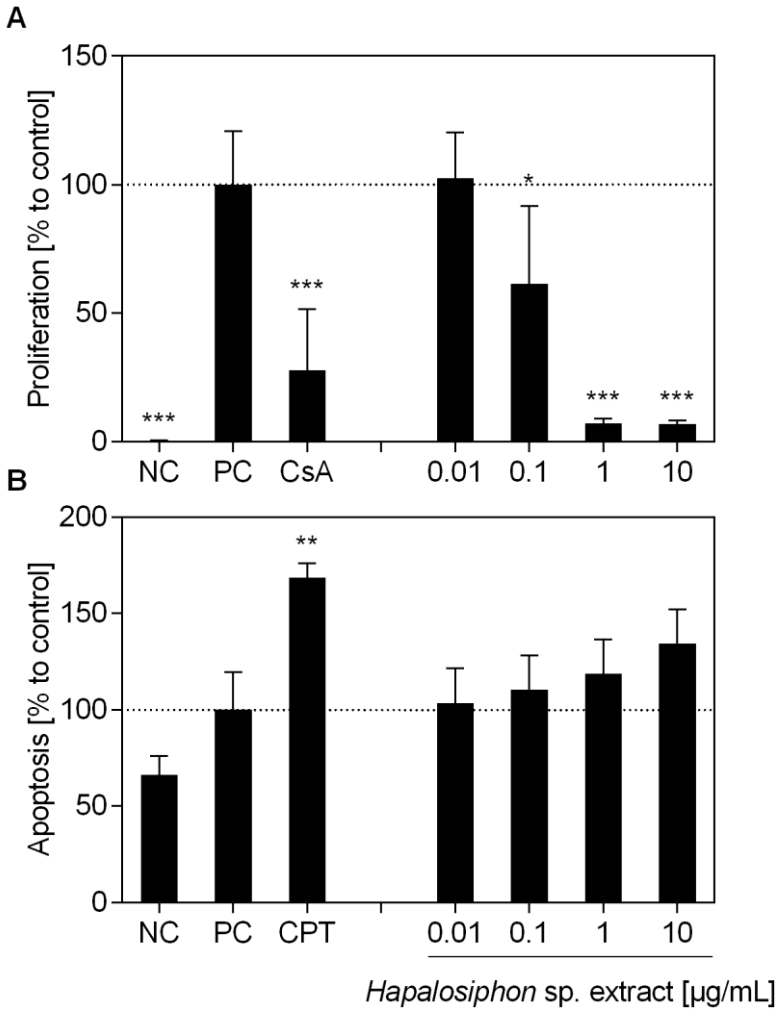
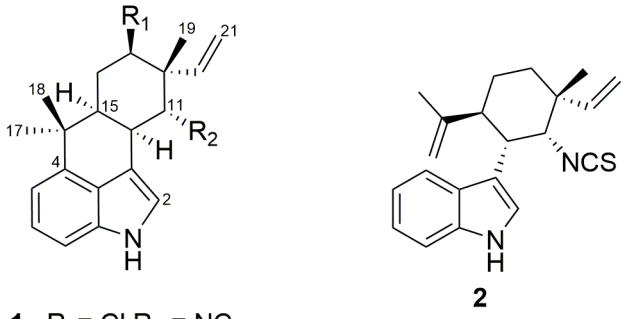


Figure 1. Effects of *Hapalosiphon* sp. CBT1235 extract on T cell proliferation and apoptosis induction. Primary human lymphocytes were cultured in the presence of medium (NC) or were stimulated with anti-human CD3 and anti-human CD28 mAb (PC; 100 ng/mL). Activated T cells were further incubated with cyclosporine A (CsA; 5 µg/mL), camptothecin (CPT; 30 µg/mL) or different concentrations of the *Hapalosiphon* sp. CBT1235 extract. Cell division analysis was carried out using CFSE staining and flow cytometry. Levels of apoptosis were determined using flow-cytometric analysis of annexin V stained cells. (**A**) Effects of *Hapalosiphon* sp. CBT1235 extract on lymphocyte proliferation. (**B**) Induction of apoptosis by *Hapalosiphon* sp. CBT1235 extract. Data of four (**A**) or three (**B**) independent experiments are presented as mean ± SD in relation to stimulated T cells (PC = 100%). Asterisks indicate significant differences from PC controls (**$p < 0.01$, ***$p < 0.001$).

Five compounds present in the active micro-fraction were isolated from the extract by semi-preparative HPLC (**1-5**). ^{1}H NMR spectra of the pure compounds displayed typical signals of hapalindoles. The molecular formulas were deduced from HRESIMS data. The

structures of **1-5** were elucidated based on 1D and 2D NMR data. NMR and MS spectral data of compounds **1**-**3** matched the published data for hapalindole A (**1**), hapalindole D (**2**), and hapalindole M (**3**), respectively (structures of all isolated compounds see **Fig. 2**). NOESY experiments as well as the determination of specific rotation values confirmed the absolute configuration of compounds **1**-**3** as originally described in detail by Moore *et al*. [10]. Hapalindole A (**1**) was the main compound isolated from *Hapalosiphon sp.* CBT1235.



2

1 R_1= Cl R_2 = NC
3 R_1= H R_2 = NCS
4 R_1= Cl R_2 = NHCHO
5 R_1= H R_2 = NHCHO

Figure 2. Chemical structures of hapalindoles **1-5** isolated from *Hapalosiphon* sp. CBT 1235.

Compounds **4** and **5** were hapalindole derivatives not yet described as natural products. Compound **4** was isolated as a brown oil. HRESIMS showed an $[M+H]^+$ ion with *m/z* 357.1723, corresponding to the molecular formula $C_{21}H_{25}N_2OCl$ (calcd. 357.1728, Δ 1.54 ppm). The ^{1}H-spectrum of **4** was almost identical with the respective spectrum of **1** but with two additional peaks in the low-field region (8.04 ppm and 8.57 ppm) and a deshielded H-11 (4.84 ppm) compared to **1** (4.37 ppm), suggesting C-11 to be substituted with a formamide moiety (**Table 1**). Key HMBC and COSY correlations confirmed the structure (**Fig. 3**). Evaluation of the NOESY spectrum showed the relative configuration of **4** to be the same as hapalindole A with the formamide group being attached axially to C-11 (strong correlations of the N-formamide proton with H-10, H-13 and H-15) [9]. Comparing the specific rotation values with already published data confirmed our assignment of the absolute configuration of **4** [37]. Therefore, compound **4** was identified as the new natural product Hapalindole A-formamide.

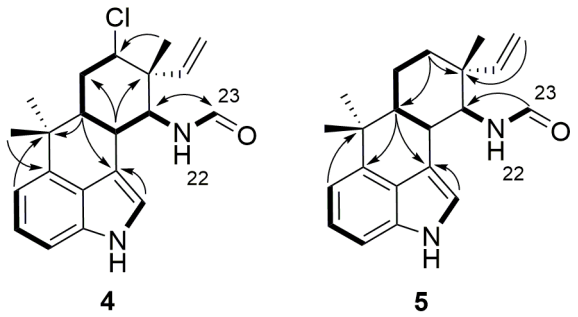


Figure 3. ^{1}H-^{1}H COSY (bold connections) and selected HMBC correlations (arrows) of **4** and **5**.

The main difference with **5** compared to **1** and **4** was the absence of the chlorine. Compound **5** was also isolated as a brown oil. HRESIMS showed an $[M+H]^+$ ion with *m/z* 323.2114, corresponding to the molecular formula $C_{21}H_{26}N_2O$ (calcd. 323.2118, Δ 1.08 ppm). Evaluation of the NMR spectra confirmed the presence of a formamide group at C-11 of the hapalindole backbone, as well (**Fig. 3** and **Tab. 1**). The absolute configuration of **5** matches the one of hapalindole J, the non-chlorinated variant of hapalindole A (**1**), and has been confirmed by comparing the NOESY correlations and the specific rotation values with those originally published by Moore *et al*. [10]. Additional NOESY correlations for the axial formamide could be observed (**Fig. 4**). Compound **5** was thus confirmed to be the new natural product Hapalindole J-formamide.

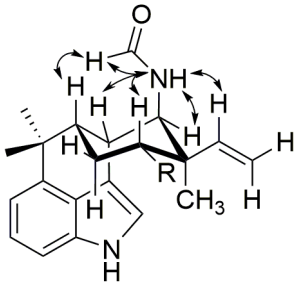


Figure 4. Selected NOESY correlations (arrows) of compounds **4** (R = Cl) and **5** (R = H).

Coupling constants between H-22 (NH) and H-23 were found to be 1.37 Hz and 1.45 Hz for hapalindole A-formamide and J-formamide, respectively, indicating a *Z* configuration for both compounds [31].

Hapalindole formamides have been described as intermediates during the total synthesis of hapalindoles, as derivatisation products with formic acid, and they can also form during

storage in *d*-chloroform [13, 31, 32, 38]. Here, we report for the first time the isolation of two hapalindole formamides as natural products from *Hapalosiphon* sp. CBT1235. **4** and **5** could readily be detected by HPLC-MS in a fresh *Hapalosiphon* sp. CBT1235 extract that has not been in contact with formic acid or other acids (**Fig. 2S**), ruling out the possibility that the isolated formamides are processing or isolation artifacts.

Table 1. ^{1}H and ^{13}C NMR assignments for **4** and **5** (600 MHz for ^{1}H, 150 MHz for ^{13}C, DMSO-d_6). ^{13}C chemical shifts for **5** were extracted from the 2D spectra.

	4		**5**	
Position	**δ_H (J in Hz)**	**δ_C**	**δ_H (J in Hz)**	**δ_C**
1	10.81, s	indole N	10.69, s	indole N
2	7.24, t (1.8)	120.5	7.13, m	119.7
3		110.6		111.8
4		137.1		138.0
5	6.81, d (7.0)	112.3	6.79, br d (7.02)	111.9
6	7.02, t (7.6)	121.9	6.99, t (7.4)	121.5
7	7.14, d (7.9)	108.7	7.10, d (8.0)	108.1
8		133.3		133.4
9		124.0		124.4
10	3,29	36.7	3,30	36.3
11	4.84, m	54.6	4.59, s	51.6
12		44.6		39.1
13 ax	4.61, dd (12.4, 4.0)	65.3	1.74, td (13.2, 3.5)	30.2
13 eq			1.27, m	
14 ax	1.26, m	31.3	0.85 m	19.3
14 eq	1.96, td (13.0, 3.4)		1.60, br d (10.5)	
15	2.11, br td (13.1, 3.8)	44.4	1.87, m	43.4
16		37.4		37.3
17	1.46, s	24.3	1.43, s	24.5
18	1.04, s	31.9	1.06, s	31.5
19	0.85, s	19.6	0.74, s	26.4
20	5.82, dd (17.1, 11.0)	144.2	5.87, m	147.7
21	5.08, m	114.0	4.87, m	109.9
22	8.57, br d (9.9)	N	8.23, br d (9.16)	N
23	8.04, d (1.37)	159.7	8.00, br d (1.45)	159.6

All isolated hapalindoles showed significant effects in the T cell proliferation assay (**Fig. 5**). Hapalindole A (**1**) displayed the highest anti-proliferative activity with an IC_{50} of 1.56 µM. Hapalindoles D (**2**) and M (**3**) showed a weaker activity and suppressed proliferation

with an IC_{50} value of 27.15 µM for hapalindole D. Hapalindoles A-formamide (**4**) and J-formamide (**5**) again showed lower activity (**Fig**. **5**). Except for hapalindole J-formamide, all hapalindoles induced T cell apoptosis at highest concentrations (**Fig**. **6**).

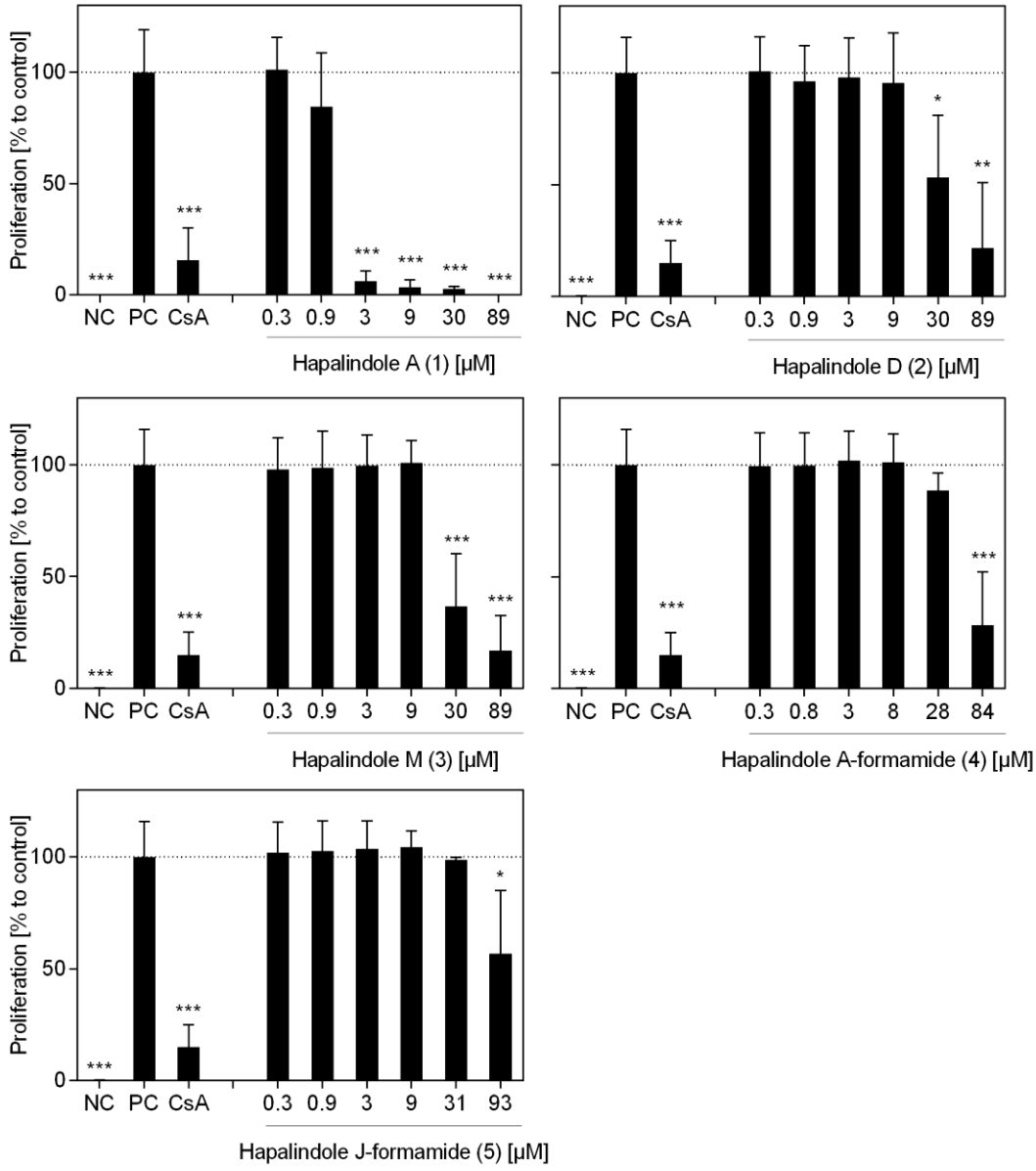


Figure 5. Effects of different hapalindoles on T cell proliferation. Primary human lymphocytes were cultured in the presence of medium (NC) or were stimulated with anti-human CD3 and anti-human CD28 mAb (PC; 100 ng/mL). Activated T cells were further incubated with cyclosporine A (CsA; 5 µg/mL) or different concentrations of **1** - **5**. Cell division analysis was carried out using CFSE staining and flow cytometry. Data of three independent experiments are presented as mean ± SD in relation to stimulated T cells (PC = 100%). Asterisks indicate significant differences from PC controls (**p < 0.01, ***p < 0.001).

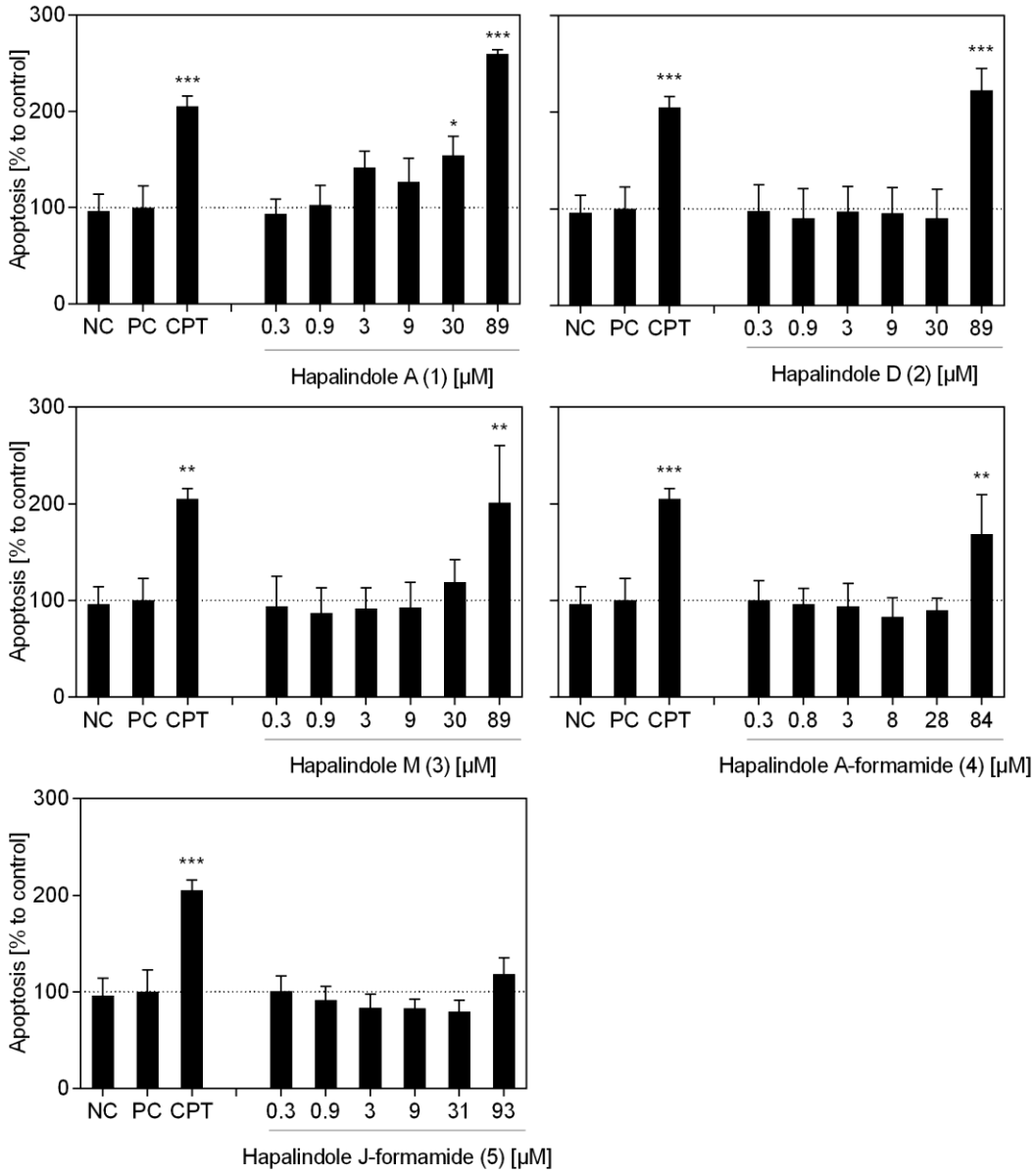


Figure 6. Levels of T cell apoptosis after treatment with different hapalindoles. Primary human lymphocytes were cultured in the presence of medium (NC) or were stimulated with anti-human CD3 and anti-human CD28 mAb (PC; 100 ng/mL). Activated T cells were further incubated with camptothecin (CPT; 30 µg/mL) or different concentrations of **1 - 5**. Levels of apoptosis were determined using flow-cytometric analysis of annexin V stained cells. Data of three independent experiments are presented as mean ± SD in relation to stimulated T cells (PC = 100%). Asterisks indicate significant differences from PC controls (**$p < 0.01$, ***$p < 0.001$).

Although relevant amounts of T cell apoptosis were detected, retardation of T cell proliferation was measurable at up to 10-fold lower concentrations. The most potent compound, hapalindole A, was effective at a concentration of 3.0 µM without showing cytotoxic effects. Therefore, potentially a therapeutic range for an application as an anti-inflammatory remedy is given. The isonitrile functional group seems to be crucial for the antiproliferative bioactivity of the hapalindoles, as the formamide derivatives possess a weaker activity. This is in agreement with previous findings, where a reduced antifungal and antibacterial activity of the hapalindole formamides compared to their isonitrile and isothiocyanate counterparts has been reported [31]. Our results, therefore, strengthen the key role of this functional group in regards to the bioactivity of members of the hapalindole family. Our work shows that anti-proliferative effects on human T cells are more pronounced than toxicity on human T cells, adding this activity to the wide range of reported bioactivities of the hapalindoles. The mode of action in human immune cells remains to be investigated. Moreover, concerning the fact that hapalindoles have been described to be neurotoxic metabolites [30], detailed studies which further discriminate toxicity and immunomodulatory effects need to be carried out in order to estimate whether or not a development as immunomodulatory drugs would be possible.

Materials and Methods

General experimental procedures

HRESIMS data were obtained using a Q Exactive Plus mass spectrometer (Thermo Fisher Scientific) coupled to an UltiMate 3000 HPLC system (Thermo Fisher Scientific). Semipreparative HPLC was conducted on an UltiMate 3000 HPLC system (Thermo Fisher Scientific). NMR spectra were either recorded at 600 MHz (^{1}H frequency) on a Bruker AV-III spectrometer using a cryogenically cooled 5 mm TCI-triple resonance probe equipped with one-axis self-shielded gradients at 300 K or at 400 MHz (^{1}H frequency) on an Agilent DD2 spectrometer. Spectra were referenced indirectly. If one-dimensional spectra were not separately recorded, ^{13}C chemical shifts were extracted from the two-dimensional spectra (compounds **1**-**3, 5**).

Cyanobacterial Material

Hapalosiphon sp. CBT1235 was taxonomically identified as *Hapalosiphon* sp. on the basis of its morphology. The strain has kindly been provided by Algenol Biotech Inc. (USA) as ABCC 804 and is deposited in the culture collection of the Cyano Biotech GmbH (Germany) under the accession number CBT 1235. The strain was cultivated in BG11 medium [39] at 28 °C under continuous light (60-80 µmol $m^{-2}s^{-1}$) in 20 L scale photobioreactors and harvested semi-continuously over a period of several weeks.

Extraction, Bioassay-guided fractionation, and Isolation of Compounds 1 – 5

Cyanobacterial cells were harvested and lyophilized. 7 g dry biomass were suspended in 100 mL 50% methanol in water (v/v), treated with an ultrasonication rod (Bandelin), and extracted on a shaker for 30 min at room temperature. After centrifugation (20 min, 10800×g), the biomass was extracted using 100 mL 80% methanol (v/v). The solutions were combined and dried *in vacuo*, yielding 0.4 g of biomass extract. For microfractionation, 4 mg of extract were suspended in 0.1 mL acetonitrile and separated into 23 fractions by HPLC using a C_{18} column (250 x 4.6 mm, 5 µm, 100 Å, Luna, Phenomenex) and 5-100 % acetonitrile-water as the mobile phase at 1 mL/min in 23 min. All fractions were tested for inhibitory activity on T cell proliferation.

For preparative isolation of the active compounds, the remaining extract was dissolved in acetonitrile and fractionated by semi-preparative HPLC using a phenyl-hexyl column (250 x 10 mm, 5 µm, 100 Å, Luna, Phenomenex) and 60-80 % acetonitrile-water as the mobile phase at 4.7 mL/min in 25 min to afford 11 fractions. Fraction 1 (t_R 6.8 min) was further purified by an additional round of semi-preparative HPLC using a phenyl-hexyl column (250 x 100 mm, 5 µm, 100 Å, Luna, Phenomenex) and 40-47 % acetonitrile-water as the mobile phase at 9.5 mL/min in 20 min to afford hapalindole A-formamide (**4**, 5.0 mg, t_R 16.5 min) and hapalindole J-formamide (**5**, 2.0 mg, t_R 15.1 min). Fraction 6 (t_R 15.2 min) was further purified by semi-preparative HPLC using a pentafluorophenyl column (250 x 100 mm, 5 µm, 100 Å, Luna, Phenomenex) and 45-63 % acetonitrile-water as mobile phase at 9.5 mL/min in 30 min yielding hapalindole A (**1**, 16.1 mg, t_R 18.4 min). No further purification was needed for fraction 8 (t_R 19.2 min) and fraction 10 (t_R 22.4 min), which corresponded to hapalindole D (**2**, 5.3 mg) and hapalindole M (**3**, 6.4 mg), respectively.

Hapalindole A (**1**): $[\alpha]^{23}_D$ -64.2° (CH_2Cl_2, c 1.2); HRESIMS (positive ion mode): *m/z* 339.1616 $[M+H]^+$; NMR spectra, see **Fig**. **3S, 4S**.

Hapalindole D (**2**): $[\alpha]^{23}_D$ +45.2° (CH_2Cl_2, c 0.31); HRESIMS (positive ion mode): *m/z* 337.1727 $[M+H]^+$; NMR spectra, see **Fig**. **5S, 6S**.

Hapalindole M (**3**): $[\alpha]^{23}_D$ -6.7° (CH_2Cl_2, c 0.45); HRESIMS (positive ion mode): *m/z* 337.1727 $[M+H]^+$; NMR spectra, see **Fig**. **7S, 8S**.

Hapalindole A-formamide (**4**): brown oil; $[\alpha]^{23}_D$ -56.2° (CH_2Cl_2, c 0.45); UV (MeOH) λ_{max} (log ε): 223 (4.38), 282 (3.60), 292 (3.52) nm; HRESIMS (positive ion mode): *m/z* 357.1723 $[M+H]^+$ (calcd. for $C_{21}H_{25}N_2OCl$, 357.1728); NMR data, see **Table 1**; NMR spectra **Fig**. **9S** – **14S**.

Hapalindole J-formamide (**5**): brown oil; $[\alpha]^{23}_D$ +12.2° ($CHCl_3$, c 0.9);UV (MeOH) λ_{max} (log ε): 224 (4.24), 282 (3.50), 292 (3.41) nm; HRESIMS (positive ion mode): *m/z* 323.2114 $[M+H]^+$ (calcd. for $C_{21}H_{26}N_2O$, 323.2118); NMR data, see **Table 1**; NMR spectra **Fig. 15S** – **19S**.

Ethics statement

Written informed consent was obtained from patients prior to blood donation for research purposes. All experiments conducted on human material were approved by the Ethics committee of the University Freiburg (55/14; February 11th, 2014).

Preparation and cultivation of human immunocompetent cells

Human peripheral blood mononuclear cells (PBMC) were isolated from the blood of adult donors obtained from the Blood Transfusion Centre (University Medical Center Freiburg). Venous blood was centrifuged on a LymphoPrep gradient (density: 1.077 g/cm^3, 20 min, 500×g, 20°C; Progen). Afterwards, cells were washed twice with medium and cell viability and concentration was determined using the trypan blue exclusion test. Cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen) supplemented with 10% heat-inactivated fetal calf serum (GE Healthcare), 2 mM L-glutamine, 100 U/mL penicillin and 100 U/mL streptomycin (Invitrogen) at 37°C in a humidified incubator with a 5% CO_2/95% air atmosphere. PBMC were additionally stimulated with anti-human CD3 (clone OKT3) and anti-human CD28 (clone 28.6) mAb (100 ng/mL; both from eBioscience). Incubation was carried out as indicated in the figure captions in the presence

of medium alone, camptothecin (CPT; 30 µg/mL; Tocris), cyclosporine A (CsA, 5 µg/mL, Sandimmun 50mg/mL, Novartis), or different concentrations of the *Hapalosiphon* sp. CBT1235 extract (0.01, 0.1, 1, 10 µg/mL), fractions (semi-quantitative using dilutions 1:250, 1:500, 1:1000, 1:2000) or various hapalindoles (0.1, 0.3, 1, 3, 10, 30 µg/mL).

Cell division tracking using CFSE

PBMC were harvested and washed twice in cold PBS (Invitrogen) and resuspended in PBS at a concentration of 5 $\times 10^6$ cells/mL. CFSE (carboxyfluorescein diacetate succinimidyl ester, 5 mM; Sigma) was diluted 1/1000 and incubated for 10 min at 37°C. The staining reaction was stopped by washing twice with complete medium. PBMC were activated as described above and cultured with the *Hapalosiphon* sp. CBT1235 extract, fractions, hapalindoles or DMSO as a solvent control for 72 h. Cell division progress was analyzed from three independent experiments with a BD FACSCalibur flow cytometer using BD CellQuest Pro Software.

Determination of apoptosis and necrosis using annexin V and propidium iodide staining

Cells were cultured as described and levels of apoptosis and necrosis were determined using annexin V-FITC apoptosis detection kit (eBioscience or BD Bioscience) according to the manufacturer's instructions. After annexin V and propidium iodide staining cells were analysed by flow cytometry. CPT and Triton-X 100 (0.5%; Sigma-Aldrich) were used as positive controls for apoptosis and necrosis, respectively.

Data analysis

For statistical analysis, data were processed with Microsoft Excel and SPSS software (IBM, Version 22.0, Armonk). Data were adjusted in relation to untreated control cells (= 100% ± SD) and values are presented as mean ± SD. Statistical significance was determined by one-way ANOVA followed by Dunnett's post hoc pairwise comparisons. P values <0.05 were considered as statistically significant (* $p<0.05$, ** $p<0.01$, *** $p<0.001$). IC_{50} and EC_{50} values were determined using GraphPad Prism 6.

Supporting information

^{1}H, ^{13}C, HSQC, HMBC and NOESY spectra, MS data as well as a chromatogram of the micro-fractionation and the results of the inhibition of T cell proliferation by different *Hapalosiphon* sp. CBT1235 fractions are available as Supporting Information.

Acknowledgments: CG and CS have been financed by the Software AG foundation and DAMUS-DONATA e.V. Funding from the Federal Ministry of Education and Research (BMBF; project ANoBIn - 16GW0115) and the German Research Foundation (DFG; INST 271/388-1) for THJN is acknowledged. We thank Andrea Porzel and Peter Schmieder for recording the NMR spectra.

Conflicts of interest: H.E. is CSO and co-owner of Cyano Biotech GmbH, D. E., is CEO and co-owner of of Cyano Biotech GmbH. The company does not have any financial interest in the research presented here. The authors dclare no conflict of interest. The funding sponsors had no role in the design, writing and publishing strategy of the study, as well as in collection, analysis or interpretation of the data.

References

[1]Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC. Marine cyanobacteria—a prolific source of natural products. Tetrahedron 2001; 57: 9347–9377

[2]Dixit RB, Suseela MR. Cyanobacteria: potential candidates for drug discovery. Antonie van Leeuwenhoek 2013; 103: 947–961

[3]Welker M, Dittmann E, Döhren H von. Cyanobacteria as a source of natural products. Methods Enzymol 2012; 517: 23–46

[4]Leao PN, Engene N, Antunes A, Gerwick WH, Vasconcelos V. The chemical ecology of cyanobacteria. Nat Prod Rep 2012; 29: 372–391

[5]Salvador-Reyes LA, Luesch H. Biological targets and mechanisms of action of natural products from marine cyanobacteria. Nat Prod Rep 2015; 32: 478–503

[6]Niedermeyer TH. Anti-infective Natural Products from Cyanobacteria. Planta Med 2015; 81: 1309–1325

[7]Dittmann E, Gugger M, Sivonen K, Fewer DP. Natural Product Biosynthetic Diversity and Comparative Genomics of the Cyanobacteria. Trends Microbiol 2015; 23: 642–652

[8]Walton K, Berry JP. Indole Alkaloids of the Stigonematales (Cyanophyta): Chemical Diversity, Biosynthesis and Biological Activity. Mar Drugs 2016; 14: 73

[9]Moore RE, Cheuk C, Patterson GML. Hapalindoles: New alkaloids from the blue-green alga Hapalosiphon fontinalis. J Am Chem Soc 1984; 106: 6456–6457

[10] Moore RE, Cheuk C, Yang X-qG, Patterson GML, Bonjouklian R, Smitka TA, Mynderse JS, Foster RS, Jones ND, Swartzendruber JK, Deeter JB. Hapalindoles, Antibacterial and Antimycotic Alkaloids from the Cyanophyte Hapalosiphon fontinalis. J Org Chem 1987; 291: 1036–1043

[11] Schwartz RE, Hirsch CF, Springer JP, Pettibone DJ, Zink DL. Unusual cyclopropane-containing hapalindolinones from a cultured cyanobacterium. J Org Chem 1987; 52: 3704–3706

[12] Moore RE, Yang X-qG, Patterson GML, Bonjouklian R, Smitka TA. Hapalonamides and other oxidized hapalindoles from Hapalosiphon fontinalis. Phytochemistry 1989; 28: 1565–1567

[13] Park A, Moore RE, Patterson GML. Fischerindole L, a new isonitrile from the terrestrial blue-green alga fischerella muscicola. Tetrahedron Lett. 1992; 33: 3257–3260

[14] Smitka TA, Bonjouklian R, Doolin L, Jones ND, Deeter JB, Yoshida WY, Prinsep MR, Moore RE, Patterson GML. Ambiguine isonitriles, fungicidal hapalindole-type alkaloids from three genera of blue-green algae belonging to the Stigonemataceae. J Org Chem 1992; 57: 857–861

[15] Stratmann K, Moore RE, Bonjouklian R, Deeter JB, Patterson GML, Shaffer S, Smith CD, Smitka TA. Welwitindolinones, Unusual Alkaloids from the Blue-Green Algae Hapalosiphon welwitschii and Westiella intricata. Relationship to Fischerindoles and Hapalinodoles. J Am Chem Soc 1994; 116: 9935–9942

[16] Huber, Moore, Patterson. Isolation of a nitrile-containing indole alkaloid from the terrestrial blue-green alga hapalosiphon delicatulus. J Nat Prod 1998; 61: 1304–1306

[17] Asthana RK, Srivastava A, Singh AP, Singh SP, Nath G, Srivastava R, Srivastava BS. Identification of an antimicrobial entity from the cyanobacterium Fischerella sp. isolated from bark of Azadirachta indica (Neem) tree. J Appl Phycol 2006; 18: 33–39

[18] Becher PG, Keller S, Jung G, Süssmuth RD, Jüttner F. Insecticidal activity of 12-epi-hapalindole J isonitrile. Phytochemistry 2007; 68: 2493–2497

[19] Mo S, Krunic A, Chlipala G, Orjala J. Antimicrobial Ambiguine Isonitriles from the Cyanobacterium Fischerella ambigua. J Nat Prod 2009; 72: 894–899

[20] Mo S, Krunic A, Santarsiero BD, Franzblau SG, Orjala J. Hapalindole-related alkaloids from the cultured cyanobacterium Fischerella ambigua. Phytochemistry 2010; 71: 2116–2123

[21] Kim H, Lantvit D, Hwang CH, Kroll DJ, Swanson SM, Franzblau SG, Orjala J. Indole alkaloids from two cultured cyanobacteria, Westiellopsis sp. and Fischerella muscicola. Bioorg Med Chem 2012; 20: 5290–5295

[22] Kim H, Krunic A, Lantvit D, Shen Q, Kroll DJ, Swanson SM, Orjala J. Nitrile-Containing Fischerindoles from the Cultured Cyanobacterium Fischerella sp. Tetrahedron 2012; 68: 3205–3209

[23] Walton K, Gantar M, Gibbs PDL, Schmale MC, Berry JP. Indole alkaloids from Fischerella inhibit vertebrate development in the zebrafish (Danio rerio) embryo model. Toxins 2014; 6: 3568–3581

[24] Raveh A, Carmeli S. Antimicrobial Ambiguines from the Cyanobacterium Fischerella sp. Collected in Israel. J Nat Prod 2007; 70: 196–201

[25] Bhat V, Dave A, MacKay JA, Rawal VH. The Chemistry of Hapalindoles, Fischerindoles, Ambiguines, and Welwitindolinones. Alkaloids: Chemistry and Biology 2014; 73:65-160

[26] Klein D, Daloze D, Braekman JC, Hoffmann L, Demoulin V. New Hapalindoles from the Cyanophyte Hapalosiphon laingii. J Nat Prod 1995; 58: 1781–1785

[27] Doan NT, Rickards RW, Rothschild JM, Smith GD, Thanh Doan N. Allelopathic actions of the alkaloid 12-epi-hapalindole E isonitrile and calothrixin A from cyanobacteria of the genera Fischerella and Calothrix. J Appl Phycol 2000; 12: 409–416

[28] Doan NT, Stewart PR, Smith GD. Inhibition of bacterial RNA polymerase by the cyanobacterial metabolites 12- epi -hapalindole E isonitrile and calothrixin A. FEMS Microbiol Lett 2001; 196: 135–139

[29] Becher PG, Jüttner F. Insecticidal compounds of the biofilm-forming cyanobacterium Fischerella sp. (ATCC 43239). Environ Toxicol 2005; 20: 363–372

[30] Cagide E, Becher PG, Louzao MC, Espiña B, Vieytes MR, Jüttner F, Botana LM. Hapalindoles from the cyanobacterium fischerella: potential sodium channel modulators. Chem Res Toxicol 2014; 27: 1696–1706

[31] Bonjouklian R, Moore RE, Patterson GML. Acid-catalyzed reactions of hapalindoles. J Org Chem 1988; 53: 5866–5870

[32] Muratake H, Natsume M. Synthetic studies of marine alkaloids hapalindoles. Part I Total synthesis of (±)-hapalindoles J and M. Tetrahedron 1990; 46: 6331–6342

[33] Schwartz E, Hirsch CF, Sesin DF, Flor JE, Chartrain M, Fromtling E, Harris GH, Salvatore MJ, Liesch JM, Yudin K. Pharmaceuticals from cultured algae. J Ind Microbiol 1990; 5: 113–124

[34] Wagner MM, Shih C, Jordan A, Williams DC. In vitro pharmacology of cryptophycin 52 (LY355703) in human tumor cell lines. Cancer chemother and pharmacol 1999; 43: 115–125

[35] Eggen M, Georg GI. The Cryptophycins: Their Synthesis and Anticancer Activity. Med Res Rev 2002; 22: 85–101

[36] Rohr J. Cryptophycin Anticancer Drugs Revisited. ACS Chem Biol 2006; 1: 747–750

[37] Fukuyama T, Chen X. Stereocontrolled synthesis of (-)-hapalindole G. J Am Chem Soc 1994; 116: 3125–3126

[38] Chandra A, Johnston JN. Total synthesis of the chlorine-containing hapalindoles K, A, and G. Angew Chem Int Ed Engl 2011; 50: 7641–7644

[39] Andersen RA. Algal culturing techniques. Burlington, MA: Elsevier Academic Press; 2005

3.3 Publication 3: "Ambigols from the Cyanobacterium *Fischerella ambigua* Increase Prodigiosin Production in *Serratia* spp"

Tomasz Chilczuk, Rita Monson, Peter Schmieder, Vesselin Christov, Heike Enke, George Salmond, Timo H. J. Niedermeyer

ACS Chemical Biology 2020, *15*, 2529–2936, DOI: 10.1021/acschembio.0c00554
The Supporting Information is available at
https://pubs.acs.org/doi/10.1021/acschembio.0c00554.

Various pathogenic bacteria use QS, a cell-cell communication mechanism, to regulate processes important for their pathogenicity. The inhibition of QS systems in pathogens using QS inhibitors has been discussed as a promising strategy to treat bacterial infections caused by multi-drug resistant bacteria. Thus, the aim of this study was to identify and characterize new QS inhibitors from cyanobacteria. A cyanobacteria extract collection was screened for inhibition of the QS regulated prodigiosin production of *S. marcescens*. No cyanobacteria extracts showed QS inhibiting activity. However, an extract of *F. ambigua* (Näg.) Gomont 108b increased prodigiosin production of *S. marcescens* drastically. Micro-fractionation of the *F. ambigua* extract showed that the major lipophilic compounds were responsible for the observed activity. Four compounds were isolated by flash chromatography and semi-preparative HPLC. Their structures were elucidated based on 1D, 2D NMR and HRMS experiments. Two isolated compounds were dereplicated as ambigol A and C, whereas two remaining compounds were new and named ambigol D and E.

Detailed characterization of the prodigiosin-promoting activity of the ambigols showed that all ambigols enhance prodigiosin production in S39006. In contrast to ambigol C and E, ambigol A and D additionally exhibited antibacterial activity against S39006. Ambigol C showed the highest prodigiosin-promoting activity in this strain. Thus, extracts from S39006 cultures incubated with ambigol C were analyzed by HRMS. Prodigiosin and four homologs were identified in the extract with up to ten times higher ion intensities than the solvent control. Tandem mass spectrometry data confirmed the structure of the detected prodigiosin homologs featuring different alkyl side chain length.

A S39006 mutant with a *pigA::lacZ* fusion was used to investigate if ambigol C increases prodigiosin production by enhancing the transcription rate of the prodigiosin biosynthetic

gene cluster. Interestingly, no change in the expression of *pigA* was observed. Thus, the transcriptome of S39006 treated with ambigol C was analyzed using RNA-seq. A differential expression analysis revealed that the treatment of *Serratia* with ambigol C caused 4.8 % of the transcriptome to change. The genes *pxpA–C* were downregulated with *pxpA* showing the most pronounced alteration in gene expression of all differential expressed genes (DEGs). All three genes encode for a 5-oxoprolinase that catalyzes the hydrolyzation of 5-oxo-L-proline to L-glutamate. Subsequent HRMS analysis of extracts from S39006 cultures incubated with ambigol C identified three prodigiosin analogs with a pyrrole-2-one substructure. The incorporation of 5-oxo-L-proline instead of L-proline into prodigiosin could be caused by an attenuated catabolism of 5-oxo-L-proline in S39006, indicating the physiological role of prodigiosin as a "metabolic sink" catabolizing the overflow of metabolites from primary metabolism.

A functional enrichment analysis (KEGG) was conducted to map DEGs to biological pathways in S39006. Results of the functional enrichment analysis show that many downregulated DEGs are predicted to be involved in the assembly and maturation of the ribosomes as well as the degradation of mRNA. This data indicates that ambigols interfere with the translation machinery and associated factors for protein biosynthesis, possibly altering the proteome of S39006. Moreover, downregulated genes were enriched in the pathway "fatty acid biosynthesis", possibly causing higher concentrations of acetyl-CoA and malonyl-CoA in the cell. Malonyl-CoA is a precursor in prodigiosin biosynthesis. In addition, upregulated genes encoding for the glycine betaine ABC transporter were detected. The glycine betaine ABC transporter facilitates among others the transport of proline, possibly increasing the proline supply, another precursor of prodigiosin production, into the cell. The possible increase of proline and malonyl-CoA in S39006 caused by ambigol C treatment could be an explanation for the observed elevated prodiginine levels in the strain.

ACS chemical biology

pubs.acs.org/acschemicalbiology Articles

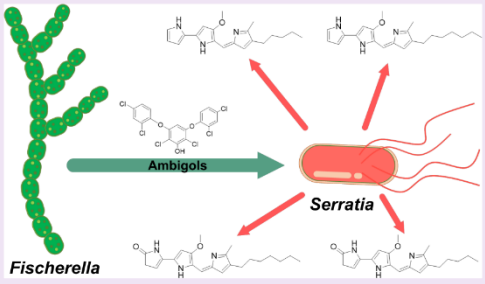
Ambigols from the Cyanobacterium *Fischerella ambigua* Increase Prodigiosin Production in *Serratia* spp

Tomasz Chilczuk, Rita Monson, Peter Schmieder, Vesselin Christov, Heike Enke, George Salmond, and Timo Horst Johannes Niedermeyer*

Cite This: *ACS Chem. Biol.* 2020, 15, 2929−2936 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: When a library of 573 cyanobacteria extracts was screened for inhibition of the quorum sensing regulated prodigiosin production of *Serratia marcescens*, an extract of the cyanobacterium *Fischerella ambigua* (Näg.) Gomont 108b was found to drastically increase prodigiosin production. Bioactivity-guided isolation of the active compounds resulted in the two new natural products ambigol D and E along with the known ambigols A and C. Ambigol C treatment increased prodiginine production of *Serratia* sp. ATCC 39006 (S39006) by a factor of 10, while ambigols A and D were found to have antibiotic activity against this strain. The RNA-Seq of S39006 treated with ambigol C and subsequent differential gene expression and functional enrichment analyses indicated a significant downregulation of genes associated with the translation machinery and fatty acid biosynthesis in *Serratia*, as well as increased expression of genes related to the uptake of L-proline. These results suggest that the ambigols increase prodiginine production in S39006 not by activating the SmaIR quorum sensing system but possibly by increasing the precursor supply of L-proline and malonyl-CoA.



Various *Serratia* species are important nosocomial pathogens that cause a range of diseases such as pneumonia, bacteremia, or wound and urinary tract infections.[1−4] The often observed multidrug resistance in different *Serratia* species and the lack of newly developed antibiotics often hamper infection treatment.[5,6] Thus, new treatment strategies are needed. Like other bacteria, some *Serratia* spp. utilize quorum sensing (QS), a cell−cell communication mechanism, to measure their population density by sensing external signals in order to regulate motility, biofilm formation, secondary metabolite production, and the production of virulence factors.[7] Interference with QS systems in pathogenic bacteria has been discussed as a promising strategy for antivirulence therapy, as this might reduce their pathogenicity and thus allow the host immune system to remove the threat before it causes too much harm.[8,9]

Several *Serratia* species also produce the QS regulated red pigment prodigiosin. Prodigiosin is a linear tripyrrole specialized metabolite with potentially beneficial properties including antimicrobial, immunosuppressive, and anticancer activity.[10,11] In *Serratia* sp. ATCC 39006 (S39006), the enzymes responsible for the production of prodigiosin are encoded by the biosynthetic gene cluster *pigA*−*O*. The bifurcated biosynthesis pathway utilizes the precursors L-proline, malonyl-CoA, L-serine, S-adenosyl-L-methionine, 2-octenal, and pyruvate to assemble the two main intermediates 2-methyl-3-pentyl-1H-pyrrole (MAP) and 4-methoxy-2,2′-bipyrrole-5-carbaldehyde (MBC). A final enzymatic condensation reaction results in the product prodigiosin (Figure S1).[12] Prodigiosin production in S39006 is governed by a complex hierarchical regulatory network[12] including a quorum sensing system (*smaI* and *smaR*).[13] Thus, cells coordinate production of prodigiosin in response to the local concentration of the signal molecule *N*-butanoyl-L-homoserine-lactone. Further known regulatory factors of prodigiosin production are transcriptional (e.g., *pigQ*, *pigR*, *pigS*, *pigV*, *pigX*, and *rap*)[14] and post-transcriptional regulators (e.g., *rsmA*, *rsmB*),[15] as well as other environmental cues such as inorganic phosphate,[16] potassium,[17] and various carbon sources.[18,19]

Although cyanobacteria have gained much attention as a prolific source for novel bioactive compounds,[20−24] little is known about QS inhibiting metabolites.[25−31] Therefore, we screened a collection of 573 cyanobacteria extracts for QS inhibition of *Serratia marcescens*. Using the inhibition of QS regulated prodigiosin production as a read-out, we found that none of the extracts reduced prodigiosin production. However, to our surprise, some extracts instead increased prodigiosin

Received: July 8, 2020
Accepted: October 20, 2020
Published: November 4, 2020

https://dx.doi.org/10.1021/acschembio.0c00554
ACS Chem. Biol. 2020, 15, 2929−2936

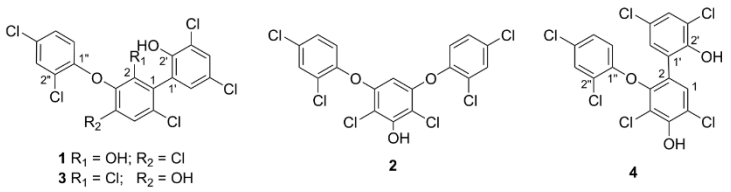


Figure 1. Chemical structures of ambigols A (**1**), C (**2**), D (**3**), and E (**4**).

production. The most active extract was derived from *Fischerella ambigua* (Näg.) Gomont 108b. Intrigued by this activity, a bioactivity-guided fractionation led to the isolation of the known ambigols A (**1**) and C (**2**) and two new natural products, ambigols D (**3**) and E (**4**). Prodigiosin biosynthesis and its regulation have been well studied in the model organism S39006.[12] Thus, this strain was selected for further characterization of the prodigiosin production promoting activity of the ambigols. All isolated ambigols increased the production of prodiginines in S39006, the most active compound, ambigol C (**2**), by up to a factor of 10. Since prodigiosin production in *Serratia* is controlled by a complex regulatory network, we conducted a transcriptome analysis to understand the influence of ambigols on the gene expression of S39006.

RESULTS AND DISCUSSION

Isolation and Structure Elucidation. Screening of 451 cyanobacteria biomass and 122 medium extracts revealed nine extracts that increased the prodigiosin production of *S. marcescens* compared to the negative control, whereas no extract caused a reduced prodigiosin production without affecting the growth of the strain (data not shown). Intrigued by the prodigiosin increasing activity of these extracts, the strain *Fischerella ambigua* (Näg.) Gomont 108b was selected for further investigation, as both the biomass and the medium extract of this strain were inducing prodigiosin production, and prominent peaks could be observed in the HPLC-DAD chromatograms of the extracts. Microfractionation and the subsequent bioassay indicated that the lipophilic compounds in fractions 14 and 16 are responsible for the increase in prodigiosin production (Figure S2). HPLC-HRMS of this extract showed that these compounds all had the same molecular formula, $C_{18}H_8Cl_6O_3$. Thus, the compounds could rapidly be dereplicated as ambigols, which had previously been isolated from this *Fischerella* strain.[32,33] The extract was subjected to flash chromatography and semipreparative HPLC, resulting in the isolation of compounds **1**−**4** (Figure 1). ^{1}H NMR analysis showed that two of the isolated compounds were ambigol A (**1**) and C (**2**; Figures S3 and S4). The ^{1}H NMR spectra of the two remaining compounds did not match literature data for ambigol B, suggesting that **3** and **4** were new ambigol derivatives (Figures S5, S6, S11, S12).[32] Their structures were elucidated based on 1D and extensive 2D NMR experiments (Figures S5−S15).

Ambigol D (**3**) was isolated as a white amorphous powder. Its molecular formula was deduced as $C_{18}H_8Cl_6O_3$ by HRMS (m/z found 480.85326 $[M - H]^-$, calculated 480.85318, Δ = 0.16 ppm)—identical to all known ambigols. The ^{1}H NMR spectrum of **3** was similar to the spectrum of ambigol A (**1**), indicating **3** to be a derivative of **1**. The substitution patterns and connections between the rings could not be assigned by regular HSQC and HMBC experiments. However, 1,1-ADEQUATE and long-range HMBC experiments showed that **3** features the same basic scaffold as **1**. Long-range HMBC and 1,1-ADEQUATE correlations revealed a hydroxy group at C-4 and a chlorine substituent at C-2, confirming the structure of the new natural product ambigol D (**3**; Figure 2, Table S1).

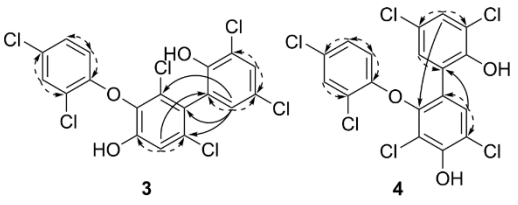


Figure 2. 1,1-ADEQUATE correlations (dashed arrows) and selected long-range HMBC correlations (bold arrows) of compounds **3** and **4**.

Ambigol E (**4**) was isolated as a white amorphous powder. Its molecular formula was also established as $C_{18}H_8Cl_6O_3$ by HRMS (m/z found 480.85314 $[M - H]^-$, calculated 480.85318, Δ = 0.09 ppm). As in the case of **3**, connections between the aromatic rings for **4** could only be assigned by 1,1-ADEQUATE and long-range HMBC experiments (Figure 2, Table S1). Both experiments confirmed the carbon−carbon bond between C-2 and C-1′ as well as the ether bridge between C-3 and C-1″, establishing a previously unreported ambigol scaffold. This novel scaffold matches the general combinatorial composition of all ambigols from 2,4-dichlorophenol as a basic monomer. The substituents at C-4 and C-5 could not be determined spectroscopically. However, since 2,4-dichlorophenol is the basic biosynthetic building block of the ambigols,[34] the two chlorine substituents must be located *meta* to each other: C-4 carries a chlorine substituent, whereas the hydroxy group must be attached at C-5. Thus, **4** was identified as the new natural product ambigol E.

Increase of Prodiginine Production by Ambigols. Ambigols **1**−**4** were tested for prodigiosin production modulating activity on S39006. S39006 was selected for further bioactivity characterization of the ambigols, as prodigiosin biosynthesis and regulation have been well studied in this strain.[12] After cultivating S39006 with and without ambigols, prodigiosin was extracted, and its relative concentration was determined spectrophotometrically. All tested compounds enhanced prodigiosin production at various concentrations after an incubation time of 10 h in S39006 (Figure 3A). Ambigol C (**2**) and E (**4**) increased prodigiosin production by 3.6- and 2.5-fold, respectively, without affecting the growth of the strain. While prodigiosin production remained on the same level when treated with concentrations of **2** that are higher than 15 μM, a decline in prodigiosin production was observed for the same concentration range after treatment with **4**. Ambigol A (**1**) and D (**3**) enhanced prodigiosin production by 1.4- and 2.4-fold at 2.0 μM and 7.8 μM, respectively. In contrast to **2** and **4**, **1** and **3**

https://dx.doi.org/10.1021/acschembio.0c00554
ACS Chem. Biol. 2020, 15, 2929−2936

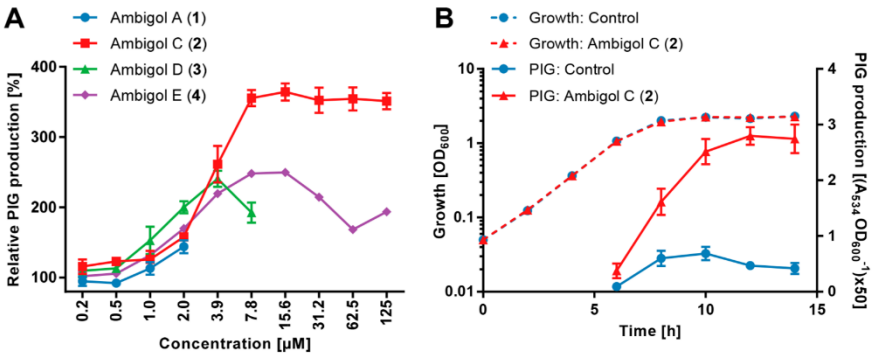


Figure 3. (A) Relative prodigiosin (PIG) production by *Serratia* sp. ATCC 39006 treated with various concentrations (0.2−125 μM) of ambigols **1**−**4**. Prodigiosin was extracted from *Serratia* cultures. The prodigiosin concentration was determined and compared to control (relative prodigiosin production). DMSO was used as a solvent control. Data are presented as mean ± SD (n = 3). (B) Effect of 15.6 μM ambigol C (**2**) and DMSO (control) on growth and prodigiosin (PIG) production of *Serratia* sp. ATCC 39006 over time. Cell growth was determined by measuring the optical density at 600 nm; n = 6. Data are presented as mean ± SD.

showed antibacterial activity against S39006 at concentrations above 3.9 μM and 15.6 μM, respectively (Figure S18). Interestingly, the antibacterial compounds **1** and **3** share the same basic scaffold, whereas **2** and **4** have a different scaffold.

To gain a better understanding of the influence of the ambigols on growth and prodigiosin production of S39006 over time, further investigations were conducted with the most active compound, ambigol C (**2**, Figure 3B). Cell growth was determined by measuring the turbidity of a S39006 culture using a spectrophotometer. No effect on the growth of the strain could be observed at 15.6 μM within 14 h. In contrast, prodigiosin production increased drastically compared to the control between 6 and 14 h.

The extracts from S39006 cultures incubated with and without 15.6 μM of **2**, sampled after 14 h, have been analyzed by HRMS. Prodigiosin (m/z 324.2070 $[M + H]^+$) and four homologues (m/z 296.1757, 310.1914, 338.2227, 352.2383 $[M + H]^+$) could be detected in the extracts with up to 10 times higher ion intensities in S39006 culture treated with **2** compared to the negative control (Figure S19). The mass difference between the prodigiosin homologues, 14 Da, indicated a different alkyl side chain length. The proposed structures were confirmed by tandem mass spectrometry data, which agreed with data from the literature (Figure S20).[35,36] Prodigiosin homologues with different alkyl chain lengths have been isolated from *Pseudoalteromonas* spp., *Serratia marcescens*, *Hahella chejuensis*, and *Zooshikella rubidus*.[35−39] The production of prodigiosin homologues is due to the substrate flexibility of prodigiosin biosynthesis enzymes. Most recently, it has been shown that PigB shows a relaxed substrate specificity, allowing for the production of the observed prodigiosin homologues.[40] In addition, PigC, which catalyzes the condensation of the two main intermediates MAP and MBC to form the product prodigiosin, is able to utilize MAP and MBC analogues as substrates, resulting in different prodigiosin homologs (Figure S1).[41,42]

Differential Expression Analysis of the Transcriptome of S39006 Treated with Ambigol C. To investigate if ambigol C induces prodigiosin production by increasing the transcription rate of the prodigiosin biosynthetic gene cluster (*pigA*−*O*), the expression of *pigA* was analyzed via a S39006 mutant with a *pigA::lacZ* fusion (strain MCP2L). To our surprise, no alteration of expression of *pigA* was detectable (Figure S21). Puzzled by this result, we set out to study the complete transcriptional changes of S39006 after exposure to ambigol C using RNA-Seq. Total RNA was isolated from the exponential phase culture of three biological replicates that were treated with and without 15.6 μM of **2** (three treated samples, three nontreated samples). Determination of the RNA integrity number (RIN) values showed a sufficient quality of all RNA samples for sequencing (Table S2). Ribosomal RNA-depleted total RNA has been sequenced on an Illumina NovaSeq, and high sequencing quality was determined for all six samples (Q30 > 84%; Table S2). Raw sequencing data were referenced against the S39006 genome with alignment rates over 89% (Table S2). A total of 214 differently expressed genes (DEGs), 4.8% of the transcriptome, were identified in the ambigol C (**2**) treatment compared to the nontreated condition by data evaluation using DeSeq2. A total of 115 genes were found to be upregulated, while 99 were downregulated (Figure 4). Transcripts with an adjusted P value <0.05 were defined as significant DEGs. A Principle Component Analysis (PCA) was conducted for all data sets to assess the reproducibility of the replicates within a condition (Figure S22). Two population clusters were identified corresponding to the two conditions (treated and untreated), indicating a good agreement among replicates for each condition. Differential expression analysis confirmed that the higher prodigiosin concentrations were not caused by elevated transcription levels of prodigiosin biosynthesis genes, since no transcripts corresponding to the genes *pigA*−*O* could be identified among the DEGs. Hence, another mode of action must be the cause of the observed prodigiosin biosynthesis enhancing activity of the ambigols.

Production of 5-Oxo-L-proline-derived Prodigiosin Analogs. Surprisingly, the observed changes in the transcriptome of S39006 after the addition of ambigol C did not reflect the observed strong influence on the prodigiosin production phenotype: The most pronounced change in expression could be observed for the gene *pxpA* (Ser39006_016520) with a $\log_2$ fold change (FC) of only −1.2. In addition, reduced transcript levels for *pxpB* (Ser39006_016505; $\log_2$ FC = −0.6) and *pxpC* (Ser39006_016500; $\log_2$ FC = −0.3) were also detected (Supporting Excel file). All three genes jointly encode an

https://dx.doi.org/10.1021/acschembio.0c00554
ACS Chem. Biol. 2020, 15, 2929−2936

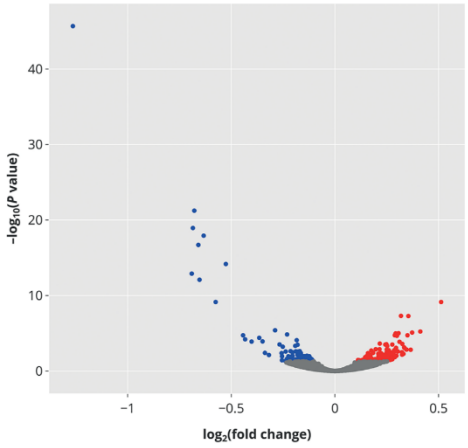


Figure 4. Volcano plot of differently expressed genes (DEGs). In total, 214 genes show different expression levels (red, expression upregulated in 115 DEGs; blue, expression downregulated in 99 DEGs; gray, no significant differential expression when comparing the ambigol C treatment with the nontreated control). An adjusted *P* value <0.05 was used to define significant DEGs.

ATP-dependent 5-oxoprolinase that catalyzes the hydrolyzation of 5-oxo-L-proline to L-glutamate.[43] Since L-proline is a precursor in prodigiosin biosynthesis (Figure S1),[44] and 5-oxo-L-proline shares a structural similarity with it, S39006 extracts were analyzed by HRMS for prodigiosin analogs with a pyrrole-2-one substructure due to incorporation of 5-oxo-L-proline instead of L-proline. Indeed, three prodigiosin analogs with a pyrrole-2-one substructure were detected in the extracts (structures confirmed by tandem mass spectrometry, Figures S20 and S23). The ion intensity of all three analogs is up to 10 times higher in the ambigol C (**2**) treated culture than in the nontreated control. The downregulation of the three genes *pxpA*−*C* suggests an attenuated catabolism for 5-oxo-L-proline in S39006. A growth inhibition effect of 5-oxo-L-proline accumulation in prokaryotes has been reported, suggesting that the prokaryotic 5-oxoprolinase might have a damage-control role.[43,45] However, no growth inhibition could be observed in S39006 culture treated with **2**, indicating either no accumulation of 5-oxo-L-proline or the existence of another catabolic pathway for 5-oxo-L-proline. A possible new catabolic pathway for 5-oxo-L-proline could be its incorporation into prodigiosin analogs through the prodigiosin biosynthesis pathway. As mentioned earlier, a substrate flexibility in the prodigiosin biosynthesis pathway has been shown that could enable the use of 5-oxo-L-proline as an alternative precursor in the biosynthesis of the observed analogs.[40−42] Indeed, prodigiosin was proposed as a "metabolic sink" for bacteria to catabolize excess metabolites from primary metabolism, such as proline.[46] However, it is difficult to explain the elevated levels of prodigiosin and homologues caused by ambigol C treatment by a downregulation of 5-oxoprolinase activity in S39006. Thus, it would be of interest to investigate the effect of *pxpA*−*C* mutants on prodigiosin production in S39006 in order to elucidate the potential physiological role of prodigiosin as a disposal system for 5-oxo-L-proline in a future study.

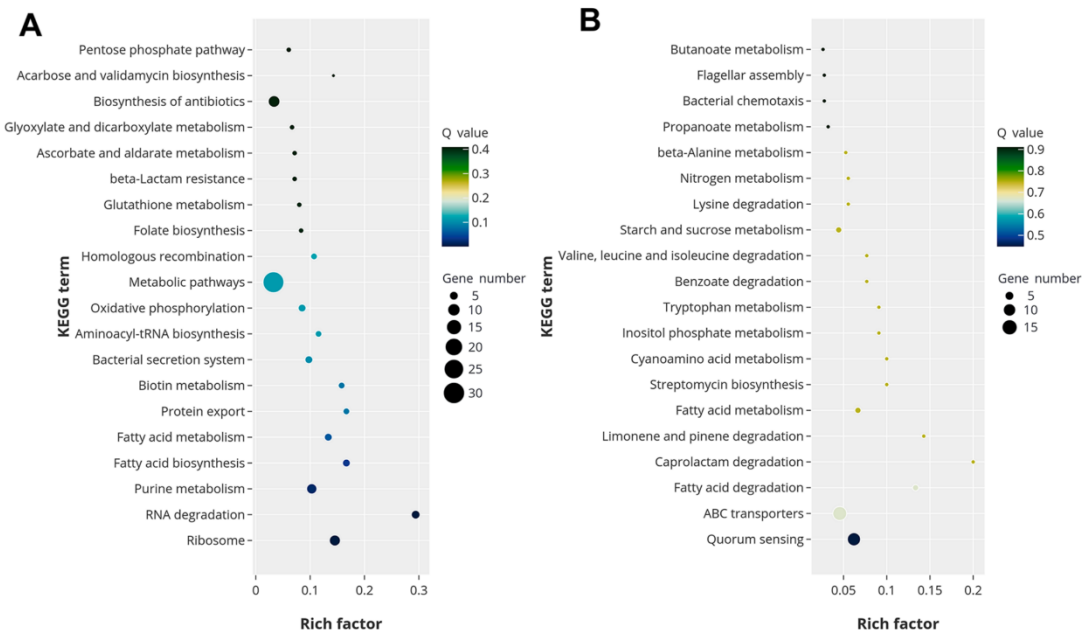


Figure 5. Scatter plots of the KEGG pathway enrichment of differently expressed genes (DEGs). (A) Top 20 KEGG pathways assigned to downregulated genes. (B) Top 20 KEGG pathways assigned to upregulated genes. The rich factor is the ratio of the DEG number to the background number in a certain KEGG pathway. The color of the dots represents the adjusted *P* value (*Q* value), the size of the dots represents the number of DEGs.

https://dx.doi.org/10.1021/acschembio.0c00554
ACS Chem. Biol. 2020, 15, 2929−2936

Functional Enrichment Analysis of DEGs. Genes that were identified as DEGs in S39006 after treatment with ambigol C were further characterized using a Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis.[47]

In total, 59 downregulated genes were assigned to 47 KEGG pathways. However, only the KEGG pathways "ribosome," "RNA degradation," "purine metabolism," and "fatty acid biosynthesis" have been significantly enriched with an adjusted P value <0.05 (Q value, Figure 5A). DEGs from the most specific KEGG pathways "ribosome" and "RNA degradation" can be associated with the translation machinery (Table S3), potentially indicating a major impact of ambigols from *Fischerella* on the protein biosynthesis and thus the proteome of *Serratia*. Downregulated genes from the KEGG pathway "ribosome" are predicted to be involved in the assembly and maturation of the ribosomes (e.g., various ribosomal proteins), whereas the downregulated genes from the KEGG pathway "RNA degradation" encode for proteins involved in the degradation of mRNA (e.g., RNA helicases, transcription termination factor, polyribonucleotide nucleotidyltransferase).

Further pathways identified by KEGG enrichment analysis are the purine metabolism and the fatty acid biosynthesis. A clear connection between downregulated genes in the purine pathways and the prodigiosin biosynthesis could not be established. However, cyclic-di-GMP and cyclic AMP have been reported as possible modulators of the prodigiosin biosynthesis and could be affected by the ambigol treatment.[48,49] A downregulation of genes encoding for proteins of fatty acid biosynthesis could lead to an increase in acetyl-CoA and malonyl-CoA concentrations in *Serratia*. Malonyl-CoA is a main precursor in prodigiosin biosynthesis, suggesting an increased precursor supply for the prodigiosin production due to ambigol treatment (Figure S1).[44]

Moreover, 25 upregulated genes were assigned to 30 KEGG pathways (Figure 5B). None of the enriched pathways for upregulated genes have an adjusted P value <0.05 (Q value). The most enriched pathway for upregulated genes is the KEGG pathway "quorum sensing." The genes *lsrA*−*R* (Ser39006_021920−Ser39006_021960) were enriched in this pathway and are among the significant DEGs. They encode for proteins involved in the LuxS-based QS system of S39006, which constitutes a two-component system for the detection and transduction of the QS signal molecule autoinducer-2.[50] A link between the LuxS-based QS system and prodigiosin biosynthesis in S39006 has not been established yet. To date, only the SmaIR QS system (LuxIR-type) has been shown to control prodigiosin production in S39006.[13] Interestingly, the LuxS-based QS system does regulate prodigiosin biosynthesis in other *Serratia* strains such as *Serratia marcescens* ATCC 274, suggesting regulatory variations for prodigiosin between strains of the same genus.[51]

Within the pathway "ABC transporters," the upregulated gene *proV* (Ser39006_007500) has been identified as a DEG. This gene encodes for one of the three subunits of the glycine betaine ABC transporter, an osmoresponsive ABC transport system for glycine betain, proline betain, and, to a lesser extent, proline. The two genes *proW* (Ser39006_007495) and *proX* (Ser39006_007490), corresponding to the other two subunits of the glycine betaine ABC transporter, also show elevated expression levels. However, they are not among the significant DEGs identified through differential expression analysis. The elevated gene expression levels of the glycine betaine ABC transporter could cause a possible increase of proline in the cell, potentially increasing prodigiosin biosynthesis precursor supply.

Potential Relationship between *Fischerella* and *Serratia* spp. The dramatic impact of ambigols produced from *F. ambigua* on the prodigiosin production of *Serratia* spp. raises the questions if the observed interaction is occurring in the environment and what ecological function it may have. A bacterial interaction between *Fischerella* and *Serratia* species has not been described so far. However, various species from both genera have been found in the same aquatic and soil environments, e.g., hot springs,[52,53] springs,[54,55] rice fields,[56,57] and lakes,[58,59] making a bacterial interaction between both organisms possible.

Several *Fischerella* and *Serratia* species possess plant growth promoting attributes. Species of both genera have the ability to fixate atmospheric nitrogen and thus increase its availability to plant roots.[57,60] Rice field isolates of *Fischerella* exhibit phosphatase activity, indicating an important ecological function in maintaining phosphate dynamics in rice fields.[61] Moreover, it has been shown that several plant-isolated *Serratia* species suppress the growth of phytopathogenic fungi by producing prodigiosin.[62,63] As both genera show symbiotic relationships with various plants (e.g., rice), the increase in production of the antifungal factor prodigiosin by *Serratia* species due to the secretion of ambigols by *F. ambigua* could be a new plant growth promoting strategy utilized by *F. ambigua*.

Furthermore, both genera are known for the production of allelochemicals that can affect the growth of other aquatic organisms.[64,65] As prodigiosin has antialgal and antibacterial activity,[10,64] the ambigols secreted by *F. ambigua* might modulate the aquatic microbiome. Given that *F. ambigua* is a benthic filamentous phototroph,[66] its prodigiosin-production promoting activity may be a strategy to prevent shading by planktonic organisms and/or deterring other organisms from colonizing its filaments. However, the possible ecological functions of the prodigiosin-promoting activity of *F. ambigua* are highly speculative. Thus, the investigation of the potential relationships between *Fischerella* and *Serratia* species needs to be the subject of future studies.

■ CONCLUSIONS

A screening for QS-modulating compounds from cyanobacteria identified several extracts that increased prodigiosin production in *Serratia* species. The ability of cyanobacterial specialized metabolites to induce prodigiosin production in *Serratia* has not yet been reported. Isolation and structure elucidation of the active compounds revealed two new ambigols from *F. ambigua* with one of them having a novel arrangement of the monomeric building blocks. The ambigols increased prodiginine production in S39006 by up to a factor of 10. The mode-of-action for this activity could not be identified unambiguously using RNA-Seq, since several levels of the translational regulation system have been altered by the treatment with ambigol C (**2**), with no clear consequences for prodiginine production in S39006. However, we present evidence for modified translation machinery in S39006 that possibly results in an altered proteome, which will be investigated in a subsequent study. The possibly increased concentrations of malonyl-CoA due to a downregulated fatty acid biosynthesis, and the potentially higher uptake of L-proline could additionally contribute to the enhanced production of prodiginines. An increase in prodiginine production due to activation of the SmaIR quorum sensing system in S39006 could not be observed. The results reported here highlight the ability

https://dx.doi.org/10.1021/acschembio.0c00554
ACS Chem. Biol. 2020, 15, 2929−2936

of *Serratia* spp. to sense small molecules from other bacteria in the local environment and modulate its physiology in response. As various strains from the genera *Serratia* and *Fischerella* colonize aquatic and soil habitats, future investigations should clarify if, and in what relationship, both strains interact with each other.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acschembio.0c00554.

Results of the differential expression analysis for all genes of S39006 (XLSX)

General experimental procedures; initial prodigiosin screening assay; cyanobacterial material; extraction and isolation of the compounds; quantification of relative prodigiosin production in *Serratia*; total RNA extraction; RNA-Seq and data analysis; ^{1}H, ^{13}C, HSQC, HMBC, and 1,1-ADEQUATE spectra; IR spectra; MS data; scheme of the biosynthetic pathway of prodigiosin; β-galactosidase activity measurement; PCA plot of differential expression analysis; results of the total RNA and the sequencing quality evaluation; overview of all DEGs that can be associate with post-transcriptional regulation mechanisms; chromatogram of the microfractionation and the *Serratia marcescens* bioassay (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Timo Horst Johannes Niedermeyer − *Department of Pharmaceutical Biology/Pharmacognosy, Institute of Pharmacy, University of Halle-Wittenberg, Halle, Germany*; orcid.org/0000-0003-1779-7899; Phone: +49/(0)345/55-25765; Email: timo.niedermeyer@pharmazie.uni-halle.de

Authors

Tomasz Chilczuk − *Department of Pharmaceutical Biology/Pharmacognosy, Institute of Pharmacy, University of Halle-Wittenberg, Halle, Germany*

Rita Monson − *Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, United Kingdom*

Peter Schmieder − *Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Department of NMR-Supported Structural Biology, Berlin, Germany*

Vesselin Christov − *Zentrum für medizinische Grundlagenforschung, University of Halle-Wittenberg, Halle, Germany*

Heike Enke − *Cyano Biotech GmbH, Berlin, Germany*

George Salmond − *Department of Biochemistry, University of Cambridge, Cambridge CB2 1QW, United Kingdom*

Complete contact information is available at: https://pubs.acs.org/10.1021/acschembio.0c00554

Author Contributions

T.C. and T.H.J.N. initiated the project. T.C. designed the experiments, performed all bioactivity assays, isolated the compounds, elucidated the structures of the compounds, isolated the RNA, and evaluated the transcriptomics data. R.M. and G.S. supervised the S39006 *pigA::lacZ* assay and supported interpretation of the transcriptomics data. H.E. cultivated the cyanobacteria and contributed the cyanobacteria extract library. T.H.J.N. and P.S. contributed to data analysis. V.C. evaluated the RNA quality. T.H.J.N. coordinated the project. T.C. and T.H.J.N. wrote the manuscript, with contributions from the other authors. All authors have given approval to the final version of the manuscript.

Funding

This work has in part been supported by the German Research Foundation (DFG; GRK 1708 (T.C.) and NI 1152/3-1 (T.H.J.N.)) and the Federal Ministry of Education and Research (BMBF, Project ANoBIn, FKZ 16GW0115). Work in the Salmond lab was supported by the BBSRC, UK (awards BB/K001833/1 and BB/N008081/1) to G.S.

Notes

The authors declare the following competing financial interest(s): H.E. is CSO and co-owner of Cyano Biotech GmbH. The company does not have any financial interest in the research presented here. The authors declare no conflict of interest. The funding sponsors had no role in the design, writing, and publishing strategy of the study, as well as in collection, analysis, or interpretation of the data.

■ ACKNOWLEDGMENTS

We thank A. Porzel for recording NMR spectra, F. Götz for providing the *Serratia marcescens* strain, and P. Janitza for his assistance with RNA-Seq data analysis. The authors also acknowledge the technical support of D. Breitmaier.

■ REFERENCES

(1) Jones, R. N. (2010) Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. *Clin. Infect. Dis. 51*, S81−S87.

(2) Blossom, D., Noble-Wang, J., Su, J., Pur, S., Chemaly, R., Shams, A., Jensen, B., Pascoe, N., Gullion, J., Casey, E., et al. (2009) Multistate outbreak of *Serratia marcescens* bloodstream infections caused by contamination of prefilled heparin and isotonic sodium chloride solution syringes. *Arch. Intern. Med. 169*, 1705−1711.

(3) Us, E., Kutlu, H. H., Tekeli, A., Ocal, D., Cirpan, S., and Memikoglu, K. O. (2017) Wound and soft tissue infections of *Serratia marcescens* in patients receiving wound care: A health care-associated outbreak. *Am. J. Infect. Control 45*, 443−447.

(4) Maki, D. G., Hennekens, C. G., Phillips, C. W., Shaw, W. V., and Bennett, J. V. (1973) Nosocomial urinary tract infection with *Serratia marcescens*: an epidemiologic study. *J. Infect. Dis. 128*, 579−587.

(5) Sandner-Miranda, L., Vinuesa, P., Cravioto, A., and Morales-Espinosa, R. (2018) The Genomic Basis of Intrinsic and Acquired Antibiotic Resistance in the Genus *Serratia*. *Front. Microbiol. 9*, 828.

(6) Projan, S. J., and Shlaes, D. M. (2004) Antibacterial drug discovery: is it all downhill from here? *Clin. Microbiol. Infect. 10*, 18−22.

(7) van Houdt, R., Givskov, M., and Michiels, C. W. (2007) Quorum sensing in *Serratia*. *FEMS Microbiol. Rev. 31*, 407−424.

(8) Clatworthy, A. E., Pierson, E., and Hung, D. T. (2007) Targeting virulence: a new paradigm for antimicrobial therapy. *Nat. Chem. Biol. 3*, 541−548.

(9) Rampioni, G., Leoni, L., and Williams, P. (2014) The art of antibacterial warfare: Deception through interference with quorum sensing-mediated communication. *Bioorg. Chem. 55*, 60−68.

(10) Lapenda, J. C., Silva, P. A., Vicalvi, M. C., Sena, K. X. F. R., and Nascimento, S. C. (2015) Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. *World J. Microbiol. Biotechnol. 31*, 399−406.

(11) Williamson, N. R., Fineran, P. C., Gristwood, T., Chawrai, S. R., Leeper, F. J., and Salmond, G. P. C. (2007) Anticancer and immunosuppressive properties of bacterial prodiginines. *Future Microbiol. 2*, 605−618.

(12) Williamson, N. R., Fineran, P. C., Leeper, F. J., and Salmond, G. P. C. (2006) The biosynthesis and regulation of bacterial prodiginines. *Nat. Rev. Microbiol. 4*, 887−899.

(13) Thomson, N. R., Crow, M. A., McGowan, S. J., Cox, A., and Salmond, G. P. (2000) Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Mol. Microbiol. 36*, 539−556.
(14) Fineran, P. C., Slater, H., Everson, L., Hughes, K., and Salmond, G. P. C. (2005) Biosynthesis of tripyrrole and beta-lactam secondary metabolites in *Serratia*: integration of quorum sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production. *Mol. Microbiol. 56*, 1495−1517.
(15) Wilf, N. M., Reid, A. J., Ramsay, J. P., Williamson, N. R., Croucher, N. J., Gatto, L., Hester, S. S., Goulding, D., Barquist, L., Lilley, K. S., et al. (2013) RNA-seq reveals the RNA binding proteins, Hfq and RsmA, play various roles in virulence, antibiotic production and genomic flux in *Serratia* sp. ATCC 39006. *BMC Genomics 14*, 822.
(16) Slater, H., Crow, M., Everson, L., and Salmond, G. P. C. (2003) Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and -independent pathways. *Mol. Microbiol. 47*, 303−320.
(17) Quintero-Yanes, A., Monson, R. E., and Salmond, G. P. C. (2019) Environmental potassium regulates bacterial flotation, antibiotic production and turgor pressure in *Serratia* through the TrkH transporter. *Environ. Microbiol. 21*, 2499−2510.
(18) Fineran, P. C., Everson, L., Slater, H., and Salmond, G. P. C. (2005) A GntR family transcriptional regulator (PigT) controls gluconate-mediated repression and defines a new, independent pathway for regulation of the tripyrrole antibiotic, prodigiosin, in *Serratia*. *Microbiology 151*, 3833−3845.
(19) Lee, C. M., Monson, R. E., Adams, R. M., and Salmond, G. P. C. (2017) The LacI-Family Transcription Factor, RbsR, Is a Pleiotropic Regulator of Motility, Virulence, Siderophore and Antibiotic Production, Gas Vesicle Morphogenesis and Flotation in *Serratia*. *Front. Microbiol. 8*, 1678.
(20) Tan, L. T. (2013) Marine Cyanobacteria: A Prolific Source of Bioactive Natural Products as Drug Leads. *Marine Microbiology*, 59−81.
(21) Niedermeyer, T. H. (2015) Anti-infective natural products from cyanobacteria. *Planta Med. 81*, 1309−1325.
(22) Demay, J., Bernard, C., Reinhardt, A., and Marie, B. (2019) Natural Products from Cyanobacteria: Focus on Beneficial Activities. *Mar. Drugs 17*, 320.
(23) Leao, P. N., Engene, N., Antunes, A., Gerwick, W. H., and Vasconcelos, V. (2012) The chemical ecology of cyanobacteria. *Nat. Prod. Rep. 29*, 372−391.
(24) Salvador-Reyes, L. A., and Luesch, H. (2015) Biological targets and mechanisms of action of natural products from marine cyanobacteria. *Nat. Prod. Rep. 32*, 478−503.
(25) Clark, B. R., Engene, N., Teasdale, M. E., Rowley, D. C., Matainaho, T., Valeriote, F. A., and Gerwick, W. H. (2008) Natural products chemistry and taxonomy of the marine cyanobacterium *Blennothrix cantharidosmum*. *J. Nat. Prod. 71*, 1530−1537.
(26) Dobretsov, S., Teplitski, M., Alagely, A., Gunasekera, S. P., and Paul, V. J. (2010) Malyngolide from the cyanobacterium *Lyngbya majuscula* interferes with quorum sensing circuitry. *Environ. Microbiol. Rep. 2*, 739−744.
(27) Kwan, J. C., Meickle, T., Ladwa, D., Teplitski, M., Paul, V., and Luesch, H. (2011) Lyngbyoic acid, a "tagged" fatty acid from a marine cyanobacterium, disrupts quorum sensing in *Pseudomonas aeruginosa*. *Mol. BioSyst. 7*, 1205−1216.
(28) Dobretsov, S., Teplitski, M., Bayer, M., Gunasekera, S., Proksch, P., and Paul, V. J. (2011) Inhibition of marine biofouling by bacterial quorum sensing inhibitors. *Biofouling 27*, 893−905.
(29) Choi, H., Mascuch, S. J., Villa, F. A., Byrum, T., Teasdale, M. E., Smith, J. E., Preskitt, L. B., Rowley, D. C., Gerwick, L., and Gerwick, W. H. (2012) Honaucins A-C, potent inhibitors of inflammation and bacterial quorum sensing: synthetic derivatives and structure-activity relationships. *Chem. Biol. 19*, 589−598.
(30) Montaser, R., Paul, V. J., and Luesch, H. (2013) Modular strategies for structure and function employed by marine cyanobacteria: characterization and synthesis of pitinoic acids. *Org. Lett. 15*, 4050−4053.
(31) Santhakumari, S., Kannappan, A., Pandian, S. K., Thajuddin, N., Rajendran, R. B., and Ravi, A. V. (2016) Inhibitory effect of marine cyanobacterial extract on biofilm formation and virulence factor production of bacterial pathogens causing vibriosis in aquaculture. *J. Appl. Phycol. 28*, 313−324.
(32) Falch, B. S., Koenig, G. M., Wright, A. D., Sticher, O., Ruegger, H., and Bernardinelli, G. (1993) Ambigol A and B: new biologically active polychlorinated aromatic compounds from the terrestrial blue-green alga *Fischerella ambigua*. *J. Org. Chem. 58*, 6570−6575.
(33) Wright, A. D., Papendorf, O., and Konig, G. M. (2005) Ambigol C and 2,4-dichlorobenzoic acid, natural products produced by the terrestrial cyanobacterium *Fischerella ambigua*. *J. Nat. Prod. 68*, 459−461.
(34) Duell, E. R., Milzarek, T. M., El Omari, M., Linares-Otoya, L. J., Schäberle, T. F., König, G. M., and Gulder, T. A. M. (2020) Identification, cloning, expression and functional interrogation of the biosynthetic pathway of the polychlorinated triphenyls ambigol A−C from *Fischerella ambigua* 108b. *Org. Chem. Front. 7*, 3193.
(35) Lee, J. S., Kim, Y.-S., Park, S., Kim, J., Kang, S.-J., Lee, M.-H., Ryu, S., Choi, J. M., Oh, T.-K., and Yoon, J.-H. (2011) Exceptional production of both prodigiosin and cycloprodigiosin as major metabolic constituents by a novel marine bacterium, *Zooshikella rubidus* S1−1. *Appl. Environ. Microbiol. 77*, 4967−4973.
(36) Eckelmann, D., Spiteller, M., and Kusari, S. (2018) Spatial-temporal profiling of prodiginines and serratamolides produced by endophytic *Serratia marcescens* harbored in *Maytenus serrata*. *Sci. Rep. 8*, 5283.
(37) Kim, D., Lee, J. S., Park, Y. K., Kim, J. F., Jeong, H., Oh, T.-K., Kim, B. S., and Lee, C. H. (2006) Biosynthesis of antibiotic prodiginines in the marine bacterium *Hahella chejuensis* KCTC 2396. *J. Appl. Microbiol. 102*, 937−944.
(38) Fehér, D., Barlow, R. S., Lorenzo, P. S., and Hemscheidt, T. K. (2008) A 2-substituted prodiginine, 2-(p-hydroxybenzyl)prodigiosin, from *Pseudoalteromonas rubra*. *J. Nat. Prod. 71*, 1970−1972.
(39) Wang, Y., Nakajima, A., Hosokawa, K., Soliev, A. B., Osaka, I., Arakawa, R., and Enomoto, K. (2012) Cytotoxic prodigiosin family pigments from *Pseudoalteromonas* sp. 1020R isolated from the Pacific coast of Japan. *Biosci., Biotechnol., Biochem. 76*, 1229−1232.
(40) Couturier, M., Bhalara, H. D., Chawrai, S. R., Monson, R., Williamson, N. R., Salmond, G. P. C., and Leeper, F. J. (2020) Substrate Flexibility of the Flavin-Dependent Dihydropyrrole Oxidases PigB and HapB Involved in Antibiotic Prodigiosin Biosynthesis. *ChemBioChem 21*, 523−530.
(41) Chawrai, S. R., Williamson, N. R., Salmond, G. P. C., and Leeper, F. J. (2008) Chemoenzymatic synthesis of prodigiosin analogues−exploring the substrate specificity of PigC. *Chem. Commun. 16*, 1862−1864.
(42) Chawrai, S. R., Williamson, N. R., Mahendiran, T., Salmond, G. P. C., and Leeper, F. J. (2012) Characterisation of PigC and HapC, the prodigiosin synthetases from *Serratia* sp. and *Hahella chejuensis* with potential for biocatalytic production of anticancer agents. *Chem. Sci. 3*, 447−454.
(43) Niehaus, T. D., Elbadawi-Sidhu, M., de Crecy-Lagard, V., Fiehn, O., and Hanson, A. D. (2017) Discovery of a widespread prokaryotic 5-oxoprolinase that was hiding in plain sight. *J. Biol. Chem. 292*, 16360−16367.
(44) Williamson, N. R., Simonsen, H. T., Ahmed, R. A. A., Goldet, G., Slater, H., Woodley, L., Leeper, F. J., and Salmond, G. P. C. (2005) Biosynthesis of the red antibiotic, prodigiosin, in *Serratia*: identification of a novel 2-methyl-3-n-amyl-pyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in *Streptomyces*. *Mol. Microbiol. 56*, 971−989.
(45) Park, C. B., Lee, S. B., and Ryu, D. D. (2001) L-pyroglutamate spontaneously formed from L-glutamate inhibits growth of the hyperthermophilic archaeon *Sulfolobus solfataricus*. *Appl. Environ. Microbiol. 67*, 3650−3654.
(46) Hood, D. W., Heidstra, R., Swoboda, U. K., and Hodgson, D. A. (1992) Molecular genetic analysis of proline and tryptophan biosyn-

https://dx.doi.org/10.1021/acschembio.0c00554
ACS Chem. Biol. 2020, 15, 2929−2936

thesis in *Streptomyces coelicolor* A3(2): interaction between primary and secondary metabolism — a review. *Gene 115*, 5−12.
(47) Kanehisa, M., and Goto, S. (2000) KEGG: kyoto encyclopedia of genes and genomes. *Nucleic Acids Res. 28*, 27−30.
(48) Fineran, P. C., Williamson, N. R., Lilley, K. S., and Salmond, G. P. C. (2007) Virulence and prodigiosin antibiotic biosynthesis in *Serratia* are regulated pleiotropically by the GGDEF/EAL domain protein, PigX. *J. Bacteriol. 189*, 7653−7662.
(49) Kalivoda, E. J., Stella, N. A., Aston, M. A., Fender, J. E., Thompson, P. P., Kowalski, R. P., and Shanks, R. M. Q. (2010) Cyclic AMP negatively regulates prodigiosin production by *Serratia marcescens*. *Res. Microbiol. 161*, 158−167.
(50) Xavier, K. B., and Bassler, B. L. (2005) Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. *J. Bacteriol. 187*, 238−248.
(51) Coulthurst, S. J., Kurz, C. L., and Salmond, G. P. C. (2004) *luxS* mutants of *Serratia* defective in autoinducer-2-dependent 'quorum sensing' show strain-dependent impacts on virulence and production of carbapenem and prodigiosin. *Microbiology 150*, 1901−1910.
(52) Hirose, Y., Fujisawa, T., Ohtsubo, Y., Katayama, M., Misawa, N., Wakazuki, S., Shimura, Y., Nakamura, Y., Kawachi, M., Yoshikawa, H., et al. (2016) Complete genome sequence of cyanobacterium *Fischerella* sp. NIES-3754, providing thermoresistant optogenetic tools. *J. Biotechnol. 220*, 45−46.
(53) Zakeri, F., Noghabi, K. A., Sadeghizadeh, M., Kardan, M. R., Masoomi, F., Farshidpour, M. R., and Atarilar, A. (2010) *Serratia* sp. ZF03: an efficient radium biosorbent isolated from hot-spring waters in high background radiation areas. *Bioresour. Technol. 101*, 9163−9170.
(54) Grimont, F., and Grimont, P. A. *The Genus Serratia: Vol. 6: Proteobacteria: Gamma Subclass*; Springer Science & Business Media, 2006.
(55) Fiore, M. F., Genuario, D. B., da Silva, C. S. P., Shishido, T. K., Moraes, L. A. B., Neto, R. C., and Silva-Stenico, M. E. (2009) Microcystin production by a freshwater spring cyanobacterium of the genus *Fischerella*. *Toxicon 53*, 754−761.
(56) Khan, Z. U., Tahmida Begum, Z. N., Mandal, R., and Hossain, M. Z. (1994) Cyanobacteria in rice soils. *World J. Microbiol. Biotechnol. 10*, 296−298.
(57) Gyaneshwar, P., James, E. K., Mathan, N., Reddy, P. M., Reinhold-Hurek, B., and Ladha, J. K. (2001) Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. *J. Bacteriol. 183*, 2634−2645.
(58) Yang, F., Wei, H. Y., Li, X. Q., Li, Y. H., Li, X. B., Yin, L. H., and Pu, Y. P. (2013) Isolation and characterization of an algicidal bacterium indigenous to lake Taihu with a red pigment able to lyse *microcystis aeruginosa*. *Biomed. Environ. Sci. 26*, 148−154.
(59) Yilmaz Ozturk, B., Asikkutlu, B., Akkoz, C., and Atici, T. (2019) Molecular and Morphological Characterization of Several Cyanobacteria and Chlorophyta Species Isolated from Lakes in Turkey. *Turk. J. Fish. Aquat. Sci. 19*, 635−643.
(60) Vaishampayan, A., Sinha, R. P., Hader, D.-P., Dey, T., Gupta, A. K., Bhan, U., and Rao, A. L. (2001) Cyanobacterial biofertilizers in rice agriculture. *Bot. Rev. 67*, 453−516.
(61) Banerjee, M. (2007) Comparative study on phosphatase activity of four rice field cyanobacterial strains and their ecological implications. *Nova Hedw. 85*, 407−416.
(62) Someya, N., Nakajima, M., Watanabe, K., Hibi, T., and Akutsu, K. (2003) Influence of bacteria isolated from rice plants and rhizospheres on antibiotic production by the antagonistic bacterium *Serratia marcescens* strain B2. *J. Gen. Plant Pathol. 69*, 342−347.
(63) John Jimtha, C., Jishma, P., Sreelekha, S., Chithra, S., and Radhakrishnan, E. (2017) Antifungal properties of prodigiosin producing rhizospheric *Serratia* sp. *Rhizosphere 3*, 105−108.
(64) Yang, K., Chen, Q., Zhang, D., Zhang, H., Lei, X., Chen, Z., Li, Y., Hong, Y., Ma, X., Zheng, W., Tian, Y., Zheng, T., Xu, H., et al. (2017) The algicidal mechanism of prodigiosin from *Hahella* sp. KA22 against *Microcystis aeruginosa*. *Sci. Rep. 7*, 7750.
(65) Schlegel, I., Doan, N. T., de Chazal, N., and Smith, G. D. (1998) Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *J. Appl. Phycol. 10*, 471−479.
(66) Soltani, N., Siahbalaie, R., and Shokravi, S. (2010) A New Description of *Fischerella Ambigua* (Näg.) Gom. - a Multidisciplinary Approach. *Int. J. Algae 12*, 19−36.

https://dx.doi.org/10.1021/acschembio.0c00554
ACS Chem. Biol. 2020, 15, 2929−2936

4 Discussion

4.1 Tjipanazoles from the cyanobacterium *Fischerella ambigua*

In this study (publication 1), a chemistry-guided screening with the software tool HaloSeeker facilitated the isolation of five tjipanazoles from the cyanobacterium *F. ambigua*.[236] Fifteen tjipanazoles were already isolated from the cyanobacterium *Tolypothrix tjipanasensis*, with most tjipanazoles featuring a sugar moiety.[101] None of the tjipanazoles discovered in this study have a sugar moiety, indicating differences in the biosynthesis of this compound family between both strains. Moreover, two other tjipanazole-like compounds, 6-cyano-5-methoxy-12-methylindolo[2,3-*a*]carbazole (**50**) and 6-cyano-5-methoxylindolo[2,3-*a*]carbazole (**51**), were isolated from the cyanobacterium *Nostoc sphaericum* EX-5-1 due to their antiviral activity and cytotoxicity against tumor cell lines (**Figure 9**).[237] All isolated tjipanazoles share the indolo[2,3-*a*]carbazole core structure. Indolo[2,3-*a*]carbazoles can be found in a wide variety of organisms,[238] e.g. rebeccamycin (**52**) from *Lechevalieria aerocolonigenes*,[239] staurosporine (**53**) from *Lentzea albida* and *Eudistoma* sp.,[240,241] and arcyriaflavin A (**54**) from *Arcyria denudate*.[242]

We proposed a biosynthetic pathway for all currently known tjipanazoles from *F. ambigua*. Parts of the proposed biosynthetic pathway show similarities with the biosynthetic pathway of other indolo[2,3-a]carbazoles, such as **52** and **53**. [243–245] No homologies could be found for the enzymes Tjp3–6 using the sequence similarity searching tool BLAST, though.[246] In addition, there are no studies to date describing the removal of the pyrrolo[3,4-c] ring from a indolo[2,3-a]pyrrolo[3,4-c]carbazole scaffold. Thus, the enzymes Tjp3–6 could possibly be involved in the removal of the pyrrolo[3,4-c] ring. However, the cleavage of the pyrrolo[3,4-c] ring could also partly occur through nonenzymatic reactions since corresponding reactions were previously described for several cladoniamides. These are indolotryptolines that are derived from indolo[2,3-a]pyrrolo[3,4-c]carbazole intermediates. In the case of cladoniamide D–G (**55**–**58**), it has been shown that the ring opening occurs through nonenzymatic destruction of intermediates in mild alkaline conditions.[247]

Another possible role for the enzymes Tjp3–6 is their involvement in the regulation of the *tjp* biosynthetic gene cluster. Genes with regulatory function (e.g. *rebR* and *staR*) were also found in biosynthetic gene clusters of **52** and **53**.[243,248] It would be of interest to investigate in future studies the effect of *tjp3–6* deletion mutants on the tjipanazole biosynthesis in *F. ambigua* (Näg.) Gomont 108b in order to elucidate the role of the corresponding enzymes.

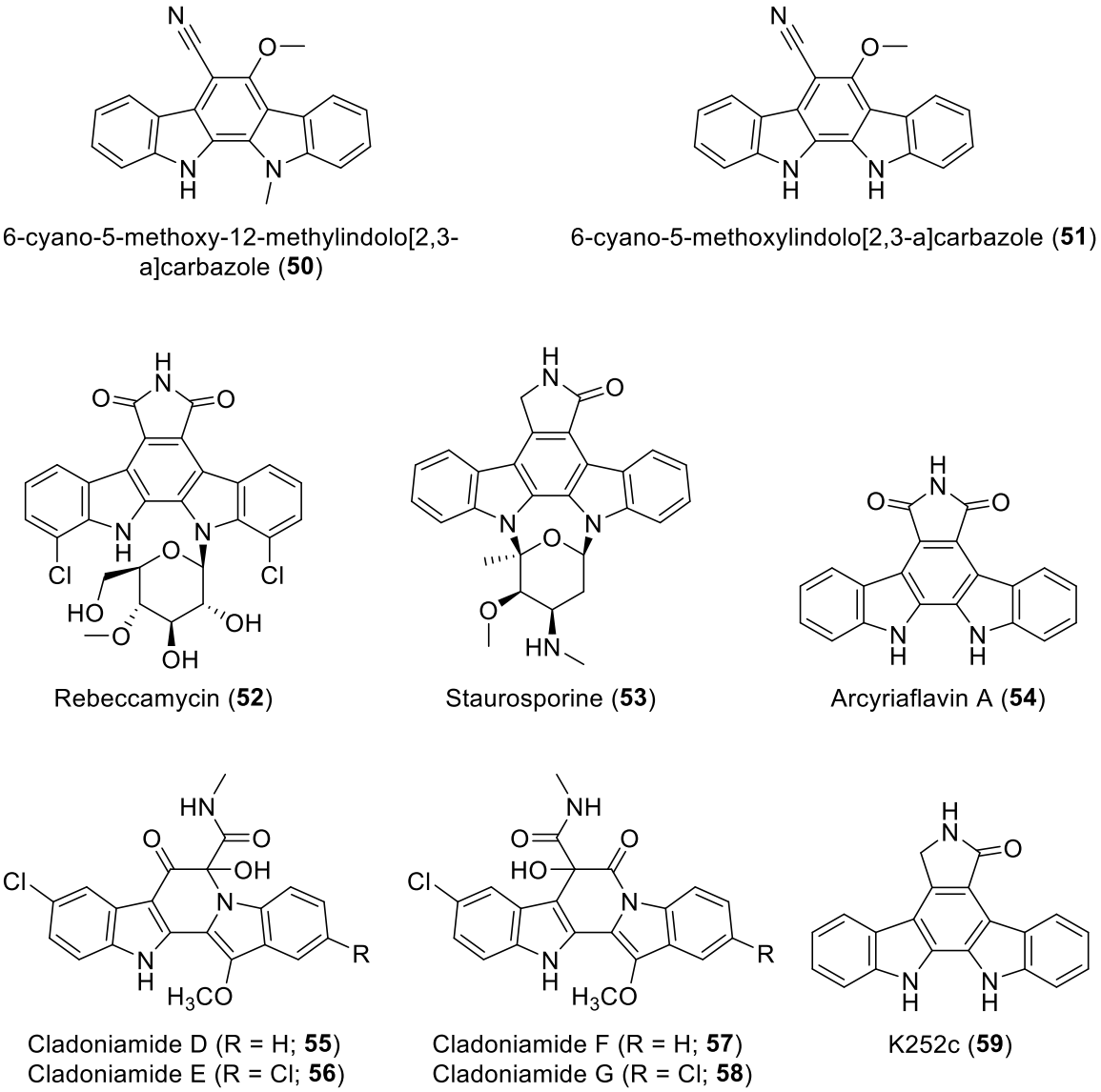


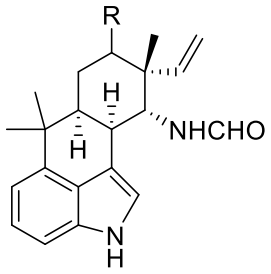
Figure 9. Structures of tjipanazole-like compounds.

The tjipanazoles isolated in this study show structural similarities with the ABCG2 inhibitors K252c (**59**) and **54**.[249] Therefore, all tjipanazoles were tested for ABCG2 inhibitory activity. Tjipanazole M, which has the highest structural similarity to arcyriaflavin A, was the most potent inhibitor from all tjipanazoles in this study. In previous studies, arcyriaflavin A exhibited antiviral activities, along with cytotoxic and apoptotic activities in cancer cells.[250,251] Moreover, it has been proven to inhibit cyclin D1-cyclin-dependent kinase 4, as well as the cell viability, proliferation, and angiogenesis of endometriotic cyst stromal cells.[252,253] Thus, future studies could investigate the influence of tjipanazoles on various viruses, cancer cell lines, as well as their effect on endometriotic cyst stromal cells.

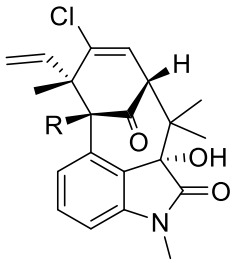
4.2 Immunomodulating hapalindoles from *Hapalosiphon* sp.

In this study (publication 2), a screening of a cyanobacteria extract collection for immunomodulating natural products facilitated the isolation of five hapalindoles from the cyanobacterium *Hapalosiphon* sp. CBT1235. Two of these, hapalindole A-formamide (**60**) and hapalindole J-formamide (**61**), were new natural products. Both compounds possess a formamide moiety, a structural feature that was found in other hapalindole-type alkaloids from cyanobacteria (**Figure 10**), including 3-hydroxy-*N*-methylwelwitindolinone C formamide (**62**),[254] fontonamide (**63**),[255] dechlorofontonamide (**64**),[256] 13-hydroxydechlorofontonamide (**65**),[257] and the hapalonamides G (**66**), H (**67**), and V (**68**).[256] Hapalonamide H (**67**) and 13-hydroxydechlorofontonamide (**65**) were tested for antimicrobial and cytotoxic activities.[257] Only **67** showed antibacterial activities against *Mycobacterium tuberculosis*, *M. smegmatis*, and *Candida albicans* as well as cytotoxic activity against vero cells. Moreover, **65** was tested against various cancer cell lines. However, no anticancer activity has been detected.[257] Hapalonamide H (**67**) and 13-hydroxydechlorofontonamide (**65**) showed lower antibacterial and anticancer activities than hapalindole A (**9**) in almost all assays.[257] In the case of *C. albicans*, **67** was more potent in inhibiting the growth of the fungi than **9**.[257]

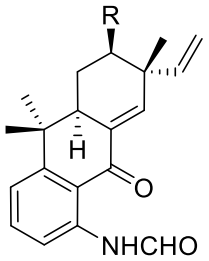
Hapalindole A-formamide (**60**) and hapalindole J-formamide (**61**) were already synthesized using a formic acid or *d*-chloroform treatment.[258–261] Both formamides exhibited weaker antibacterial and antifungal activities than hapalindoles with an isonitrile and an isothiocyanate moiety.[258]



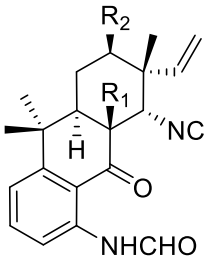
Hapalindole A-formamide (R = Cl; **60**)
Hapalindole J-formamide (R = H; **61**)



3-hydroxy-*N*-methylwelwitindolinone C formamide (R = NHCHO; **62**)



Fontonamide (R = Cl; **63**)
Dechlorofontonamide (R = H, **64**)
13-hydroxydechlorofontonamide (R = OH; **65**)



Hapalonamide G (R_1 = H, R_2 = Cl; **66**)
Hapalonamide H (R_1 = H, R_2 = H; **67**)
Hapalonamide V (R_1 = OH, R_2 = Cl; **68**)

Figure 10. Chemical structures of hapalindole-type alkaloids with formamide moiety and 3-hydroxy-N-methylwelwitindolinone C isonitrile.

All isolated hapalindoles were tested for immunomodulating activities in this study. All hapalindoles inhibited T cell proliferation, **9** being the most potent inhibitor. In addition, **9** induced T cell apoptosis. However, attenuation of T cell proliferation was detected at ten times lower concentrations. Hapalindole A-formamide (**60**) and hapalindole J-formamide (**61**) showed weaker inhibition of T cell proliferation than hapalindole derivatives with an isonitrile moiety. This is in agreement with reports showing that the hapalindole formamides or other hapalindole-type alkaloids with formamide moiety have reduced bioactivity compared to hapalindoles bearing an isonitrile or an isothiocyanate moiety.

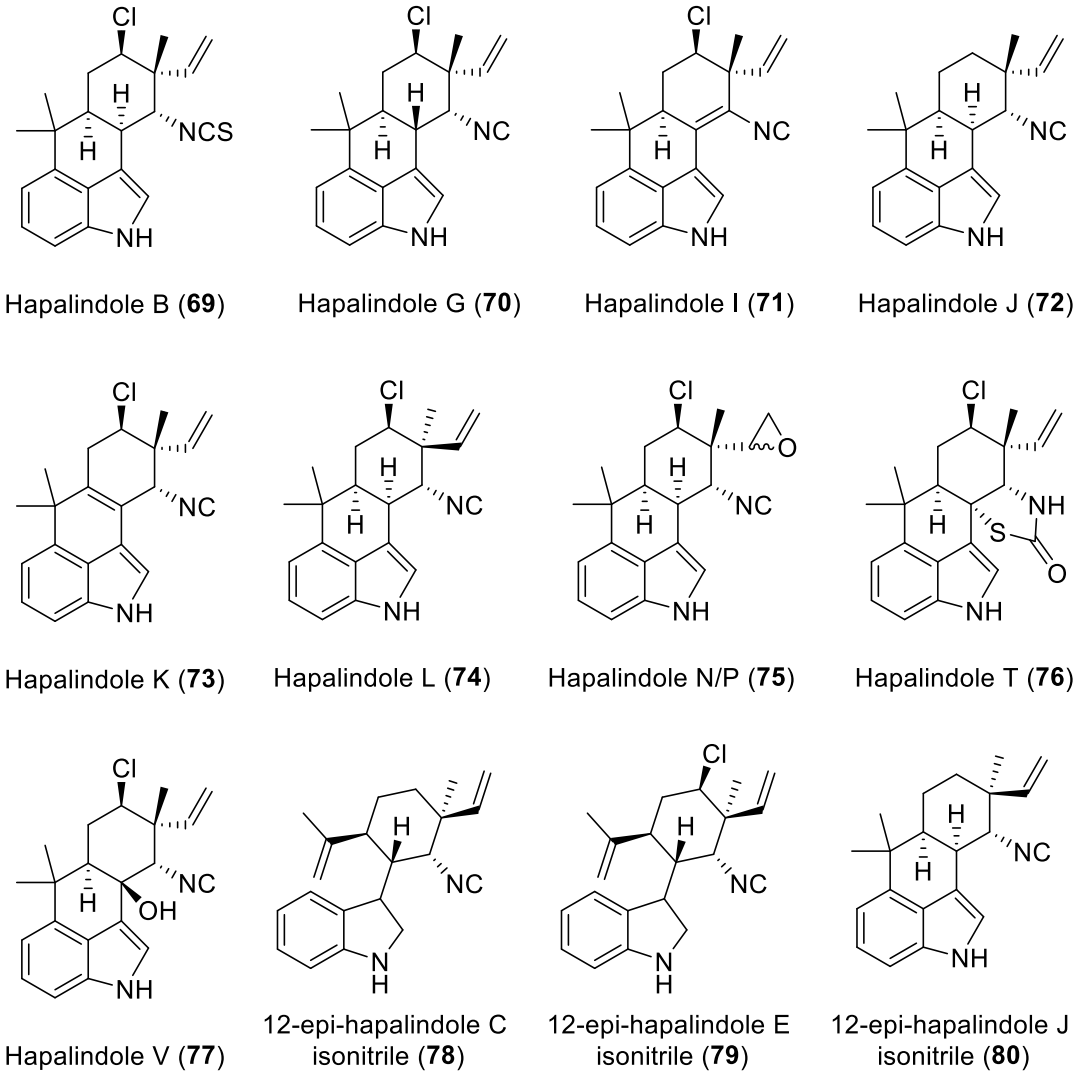


Figure 11. Chemical structures of various hapalindoles.

Our study is the first report of the immunomodulating activity of hapalindoles. A structure-activity relationship study with hapalindole derivatives of **9** should be considered to identify the pharmacophore responsible for the immunomodulating activity. Hapalindole B, G, I, J, K, L, N/P, T, and V (**69**–**77**) deviate in one structural feature from **9**, making them interesting candidates for a structure-activity relationship study (**Figure 11**). Moreover, it has been shown that 12-epi-hapalindole C isonitrile (**78**), 12-epi-hapalindole E isonitrile (**79**), 12-epi-hapalindole J isonitrile (**80**), and hapalindole L (**74**) are neurotoxins acting on sodium channels.[262] Thus, further investigations need to be conducted in order to rule out a neurotoxic activity of **9**.

4.3 Ambigols and related structures

In this study (publication 3), a screening for QS inhibitors from cyanobacteria resulted in the isolation of ambigols A (**11**), and C–E (**Figure 12**, **81**–**83**). Ambigols exhibit a wide range of bioactivities. Ambigol A (**11**) and B (**84**) proved to be potent inhibitors of the cyclooxygenase.[263] Moreover, ambigol A–C (**11**, **84, 81**) showed antibacterial and antifungal activities.[263,264] The antibacterial activity of the ambigols is more pronounced against Gram-positive bacteria rather than against Gram-negative bacteria. Further biological studies revealed cytotoxic, molluscicidal, and antiviral activities of **11** and **84**. In a brine shrimp assay, **11** and **84** showed lethal effects, possibly due to their cytotoxic activities. Interestingly, in almost all biological assays, **84** exhibited a weaker activity than **11**.[263] Furthermore, **11** and **81** showed antiplasmodial and trypanocidal activities against parasites, such as *Plasmodium falciparum* and *Trypanosoma rhodesiense*.[264]

Ambigol A–C (**11**, **84, 81**) were previously isolated from *F. ambigua*,[264,265] the only producer strain of this compound family known so far. All ambigols feature three polychlorinated phenol units linked either via biaryl- or biaryl-ether-bonds. Their structures show similarities to the structure of polybrominated diphenyl ethers (PBDEs) that either have an anthropogenic origin or are produced by marine invertebrates and marine bacteria.[266–269] Several PBDEs, e.g. 2-OH-BDE68 (**85**), have been isolated from sponges, with *Lamellodysidea* being the most prominent source (formerly known as *Dysidea*).[267,270–272] However, metagenomics studies showed that some PBDEs are in fact produced by cyanobacterial symbionts (e.g. *Hormoscilla spongeliae*) of the *Lamellodysidea* sponges, and not by the host itself.[269] It has been shown that *Pseudoalteromonas* species also produce various PBDEs.[268] Interestingly, *Pseudoalteromonas* species are often found in association with marine eukaryotic hosts, such as mussels, pufferfish, tunicates, sponges, and marine plants.[273]

PBDEs are known for their persistence in marine environments. They bioaccumulate in marine animals and were already detected in human tissues,[274–277] indicating that these compounds are likely to transfer to the human food web. A wide range of bioactivities have been reported for PBDEs including antibacterial, antifungal, cytotoxic, and various enzyme inhibition activities.[267,278–280]

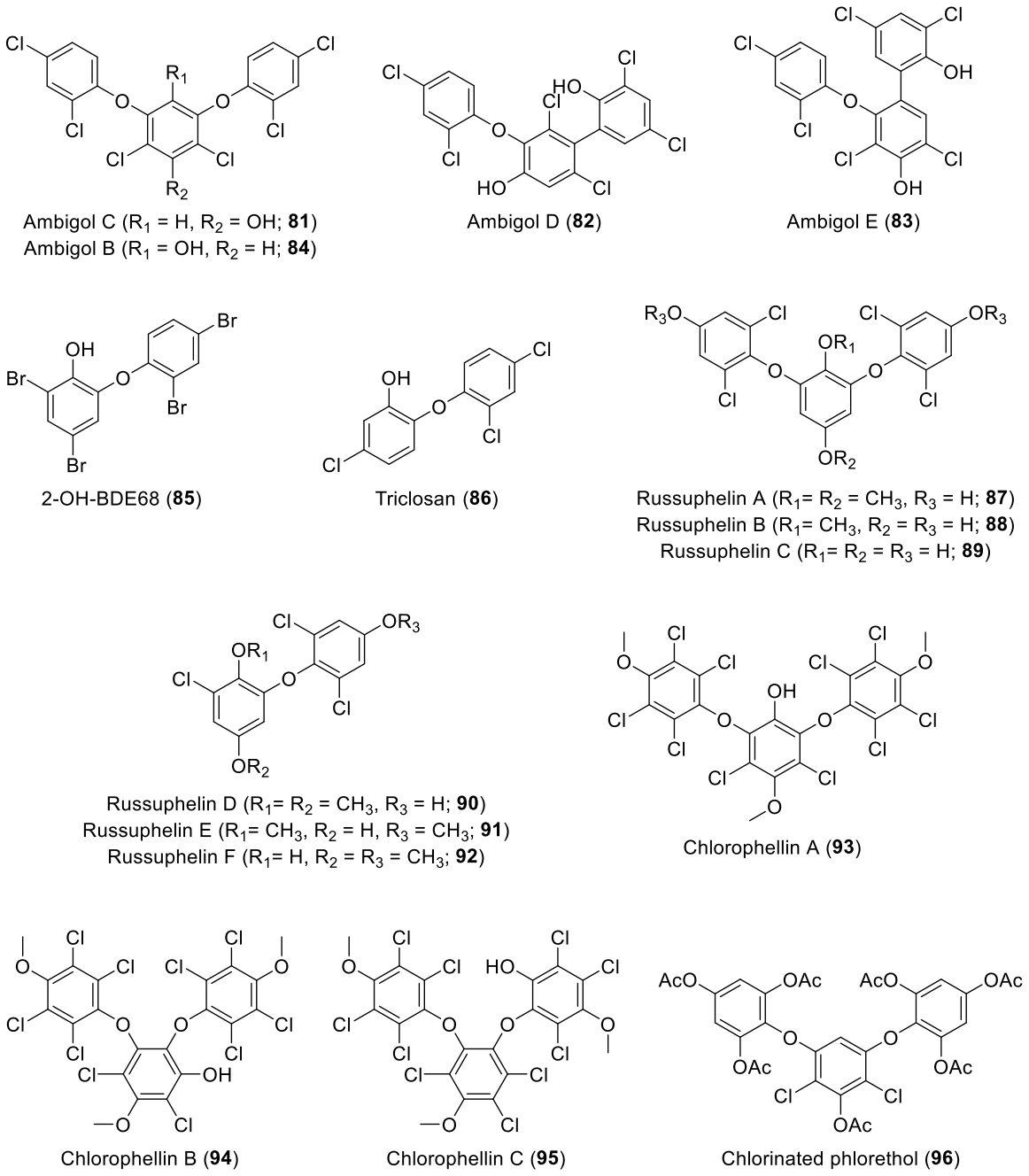


Figure 12. Structures of ambigols and chemically related compounds

Triclosan (**86**) is a polychlorinated phenoxyphenol that shares structural similarities with the ambigols. This compound was synthesized in the 1960s and is widely used in commercial and healthcare products due to its broad-spectrum antimicrobial activities.[281] Triclosan (**86**) has also been isolated from the sponge-associated bacterium *Micrococcus luteus*.[282] Its antibacterial effect is based on the inhibition of the fatty acid biosynthesis

enzyme enoyl–acyl carrier protein reductase.[283] Furthermore, **86** has been proven to be active against various cancer cell lines.[284–286] In the case of rat mammary carcinogenesis, it has been shown that **86** reduced tumor incidence and tumor numbers per animal by inhibiting the fatty acid biosynthesis.[287]

Additional ambigol-related structures are the russuphelins A–F (**87**–**92**) from *Russula subnigricans* and the chlorophellins A–C (**93**–**95**) from *Phellinus ribis*.[288–290] Both organisms are fungi. *Russula subnigricans* has been responsible for mushroom poisoning incidents in Taiwan,[291] Japan,[292] and Korea,[293] whereas *Phellinus* species have been used as traditional medicine against various diseases.[294] Russuphelins A, B, C, and D (**87**–**90**) exhibited cytotoxic activity *in vitro* against P388 leukemia cells.[288,289] During a screening for agonists of peroxisome proliferator-activated receptors (PPARs), all chlorophellins activated PPAR-γ, while **95** showed the most agonistic effect. PPARs play a role as regulators of lipid and lipoprotein metabolism, glucose homeostasis, cellular differentiation, and the inflammatory response. Thus, they are seen as a therapeutic target for the treatment of type 2 diabetes, various cancers, and inflammatory diseases.[295,296] Moreover, chlorinated phlorotannins, e.g. chlorinated phlorethols (**96**), that show a structural resemblance to ambigols were isolated from various brown algae including *Cystophora reflexa*, *Carpophyllum angustifolium*, *Analipus japonicas*, and *Laminaria ochroleuca*.[297–300] Phlorotannins are known for their antidiabetic,[301] antioxidant,[302,303] anti-inflammatory,[304] antibacterial,[305] and anticancer activities.[306,307] Due to their beneficial properties, there is a growing interest for the use of algae in food, pharmaceutical, and cosmetic applications.[308–310]

4.4 Disruption of oxidative phosphorylation by ambigol-related structures

Our study is the first report showing the ability of cyanobacterial specialized metabolites to induce prodigiosin production in *Serratia* species. The treatment of S39006 with ambigol C increased its prodigiosin production by the factor of 10. Unfortunately, the mode-of-action for this activity could not be identified unambiguously using RNA-Seq. Thus, the following section presents various studies investigating the mode-of-action of ambigol-related structures. Several ambigol-related structures including halogenated phenols and halogenated diphenyl ethers have been shown to possess uncoupling activities in various organisms (**Figure 13**). Uncoupling agents or uncouplers are compounds that

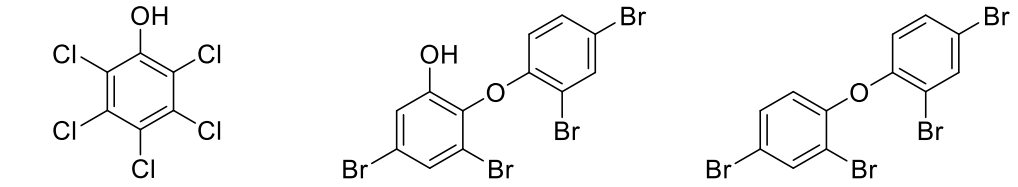
disrupt the oxidative phosphorylation by perturbing the electrochemical gradient and/or by inhibiting components of the electron transport chain or ATP synthase. The electrochemical gradient can be dissipated by biomolecules (e.g. protonophores, ionophores, membrane active peptides) that transport protons across membranes or interfere with the membrane structure.[311,312] The disruption of the oxidative phosphorylation can lead to alterations in ATP synthesis,[313,314] oxygen consumption rate,[314] heat production,[315,316] and membrane potential.[311,312,317]

Various chlorophenols that show structural similarities to ambigols such as pentachlorophenol (**97**) are considered to be protonophoric uncouplers.[318] They have the ability to translocate protons across a lipid bilayer membrane. The uncoupling activity of chlorophenols increases with the addition of chlorine substitutes.[318] Characteristic properties of protonophoric uncouplers include being weak lipophilic acids, with bulky hydrophobic groups (e.g. phenyl group), an acidic dissociable group (e.g. hydroxyl group) and strong electron withdrawing substituents (e.g. chlorine atoms).[311] Pentachlorophenol (**97**) as well as other chlorophenols have an influence on the chemotaxis and inhibit the amino acid transport (e.g. glycine and proline) of *Bacillus subtilis*.[319–321] Moreover, various chlorophenols inhibit the proton-sugar symport in Saccharomyces fragilis,[322] and show an effect on RNA, protein, and ribosome biosynthesis of different *Saccharomyces* species.[323,324] Interestingly, in our current study, the expression of genes involved in chemotaxis, proline/glycine transport, sugar transport, as well as ribosomal genes also underwent changes in S39006 following **81** exposure.

The above mentioned PBDEs also show uncoupling activity. Several PBDEs including 6-OH-BDE47 (**98**) are as potent as the uncoupler pentachlorophenol (**97**).[317] It has been shown that PBDEs disrupt the oxidative phosphorylation in zebrafish embryos by protonophoric uncoupling and/or inhibition of the electron transport chain.[325] Interestingly, a microarray analysis showed that **98** subtly altered expressions of genes involved in proton transport and carbohydrate metabolism, although **98** exhibited dramatic toxic effects *in vivo*.[326] This was explained by the mechanism of action of **98**. Protonophoric uncouplers operate their toxic effect on the membrane level instead of the DNA, RNA, and protein level. Thus, a proper feedback loop on the gene expression level could be missing.[326] In contrast, BDE47 (**99**) altered the transcriptome in mice drastically.[327] In several studies, **99** showed an influence on the expression of ribosomal genes in mice and

rats.[327–329] BDE47 (**99**) increased the expression of various ribosomal genes, whereas **81** downregulated the expression of several ribosomal genes in S39006.

Similar results have been observed for **86**. It has been shown that **86** can impair the oxidative phosphorylation in living cells and organisms via acting as a protonophore and a complex II inhibitor of the electron transport chain.[314,330–333] The effect of **86** exposure to the transcriptome of *Escherichia coli* and *Salmonella enterica* altered the expression of fatty acid biosynthetic genes and ribosomal genes in both strains.[334] The expressions of various fatty acid biosynthesis and ribosomal genes were also downregulated in S39006 treated with **81**.



Pentachlorophenol (**97**) 6-OH-BDE47 (**98**) BDE47 (**99**)

Figure 13. Chemical structures of pentachlorophenol and polybrominated diphenyl ethers that can disrupt the oxidative phosphorylation in various organisms.

All ambigols show remarkable structural similarities with various uncoupling agents like chlorophenols as well as halogenated diphenyl ethers. Moreover, in many cases transcriptome analyses of organisms exposed to chlorophenols and halogenated diphenyl ethers show similar results to the results of our transcriptome study. Thus, future studies should investigate if ambigols have the ability to disrupt oxidative phosphorylation in bacteria including *Serratia*.

Most recently, it has been shown that low cellular levels of ATP increase production of prodigiosin in *S. marcescens*.[335,336] Haddix et al. revealed that the oxidative phosphorylation inhibitor cyanide caused a depletion in cellular ATP and consequently an induction in transcription of the prodigiosin biosynthetic gene cluster.[335] Since ambigol-related structures including **98** and **86** are also uncoupling agents, ambigols could possibly increase prodigiosin production in S39006 by disrupting the oxidative phosphorylation and lowering the cellular ATP concentration as in the case of cyanide. However, our RNA-Seq data as well as data from gene expression analysis with a S39006 *pigA*::*lacZ* mutant indicate that **81** does not induce prodigiosin production by increasing

the transcription rate of the prodigiosin biosynthetic gene cluster. Hence, future studies should also investigate if ambigols improve prodigiosin biosynthesis by altering the proteome of S39006 and/or by increasing the precursor supply of prodigiosin biosynthesis.

4.5 Optimization of prodigiosin production

Our discovery of the prodigiosin-promoting activity of ambigols could be of interest for the industrial production of prodigiosin, since the prodiginine family is famous for their varied range of biological effects. Prodigiosin is regarded as a promising therapeutic drug, due to its many potential properties including anticancer, immunosuppressive, antibacterial, antiprotozoal, antifungal, and antimalarial activities.[203–207] Moreover, there is a growing interest for the utilization of prodigiosin as a biocolorant in various industries. Its potential in coloring textiles,[337,338] paper,[338,339] soap,[338] ink,[338] candles,[338,340] and polymers has been shown in multiple studies.[339,341]

Thus, a cost-effective large-scale production of prodigiosin has been subject of many investigations. Several studies focused on increasing prodigiosin production of *S. marcescens* by optimizing culture conditions, such as temperature,[342–344] pH,[345,346] media composition,[342,344,345,347] and incubation time.[342,343,347] Furthermore, it has been shown that the deletion or addition of certain genes in *Serratia* species can lead to improved prodigiosin levels. The deletion of the gene encoding for the negative regulator PigZ increased prodigiosin production in S39006,[228] whereas the addition of genes responsible for the synthesis of prodigiosin precursors elevated prodigiosin levels in *S. marcescens*.[348] Our study showed that ambigols increase prodiginine production in *Serratia* species drastically. This phenomenon has not been described yet and could be a complimentary approach in order to improve prodigiosin titer in *Serratia* for large-scale cultivation. Future structure-activity relationship studies could help identifying structural features that are essential for the prodigiosin-promoting activity of ambigols. Moreover, ambigol-related compounds including **97**, **86** and various polybrominated diphenyl ethers should be tested for prodigiosin-promoting activity.

4.6 Potential mutual relationships between *Fischerella* and *Serratia* species

The elevated prodigiosin levels in S39006 due to exposure of ambigols isolated from *F. ambigua* raises the questions if and in what relationship both strains interact with each other. Various species from both genera have been found in the same aquatic and soil environments, making a bacterial interaction between both organisms possible. Both genera were isolated from hot-springs,[349–351] springs,[182,352] rice fields,[353–360] and lakes.[361–364] The following section discusses the potential ecological function of the prodigiosin-promoting activity of *F. ambigua*. However, since there are no studies describing an interaction between *Fischerella* and *Serratia* species, the following potential ecological functions are highly speculative.

Heterocystous cyanobacteria, such as *Fischerella*, play a major role in many rice field ecosystems, due to their ability to fixate atmospheric nitrogen leading to an increase of nitrogen content in the soil.[365] In addition, it has been shown that *Fischerella* possesses phosphatase activity and therefore it has been assumed to be important in maintaining phosphate dynamics in rice fields.[366] Phosphate and nitrogen are essential macro-nutrients that are required for plant growth.[367]

Furthermore, multiple *Serratia* species proved to be endophytes in plants (e.g. rice) with plant-growth promoting activities.[358,368,369] Similar to heterocystous cyanobacteria, various *Serratia* species have the ability to fix atmospheric nitrogen and thus increase its availability to plant roots.[358,368] Moreover, Someya et al. showed that *S. marcescens* B2 suppressed the growth of *Rhizoctonia solani* presumably by secreting chitinases and prodigiosin, resulting in the protection of rice from this phytopathogenic fungus.[370] Intriguingly, the same group showed that other endophytic bacteria from rice inhibited prodigiosin production of *S. marcescens* B2.[370] In contrast, ambigols from *F. ambigua* improved prodigiosin production in S39006, demonstrating the ability of other microorganism to alter production of prodigiosin in *Serratia* species.

Various *Fischerella* and *Serratia* species are considered for application as biofertilizers, due to their promising plant-growth promoting activities.[369,371,372] Future studies should investigate if *Serratia*, *Fischerella*, and certain plants (e.g. rice) interact with each other in a mutual relationship. The co-cultivation of *Serratia* and *Fischerella* species could have a

synergistic effect on the growth of various plants, improving their potential as biofertilizers in agriculture.

Another potential mutual relationship between *Fischerella* and *Serratia* species could occur in freshwater bodies, such as lakes. *Fischerella* and *Serratia* species are known to produce allelochemicals that can inhibit or stimulate the growth of other aquatic organisms (**Figure 14**). In multiple studies, *Fischerella* species exhibited allelopathic activities against cyanobacteria including *Microcystis aeruginosa* and/or eukaryotic algae.[373–375] Several allelochemicals with algicidal activity were isolated from *Fischerella* species including fischerellin A (**100**) and B (**101**),[376,377] 12-epi-hapalindole E isonitrile (**79**),[378] and 12-epi-hapalindole F (**102**).[379] In the case of **102**, it is assumed that its algicidal activity is based on the inhibition of photosynthesis in other phototrophs.[380] Furthermore, prodigiosin has been proven to be an allelochemical against several aquatic organisms. It showed algicidal activities against the dinoflagellate *Cochlodinium polykrikoides*,[381] the haptophytes *Phaeocystis globosa*,[382,383] *P. donghaiense*,[382] the microalgae *Heterosigma akashiwo*,[382] and the cyanobacterium *M. aeruginosa*.[361,384] Interestingly, two distinct studies showed that *M. aeruginosa* inhibits prodigiosin production in *S. marcescens*,[385,386] although, the mechanism causing the anti-pigmentation activity has not been determined yet.

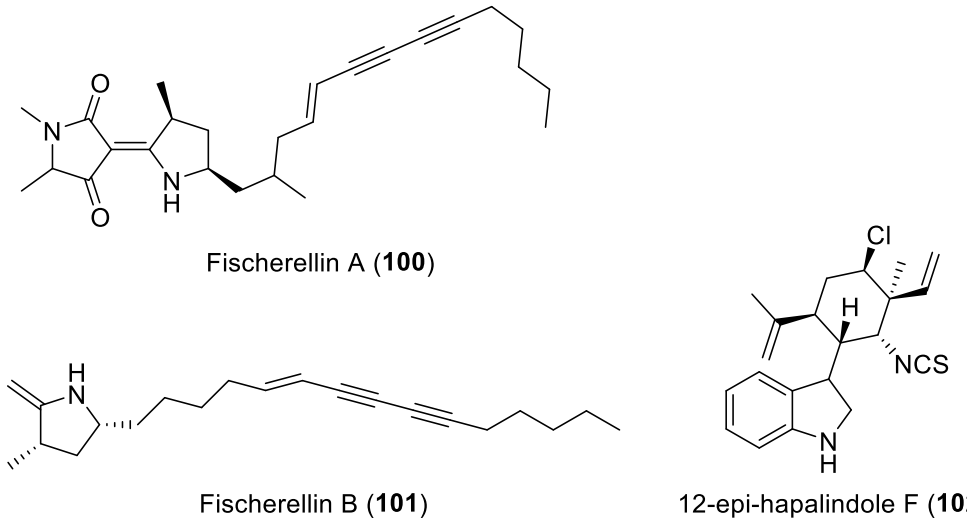


Figure 14. Chemical structures from allelochemicals with algicidal activity isolated from *Fischerella* species.

Many toxic species that are sensitive to the algicidal agents produced by *Fischerella* and *Serratia* sp. grow rapidly in water bodies leading to the phenomenon called harmful algal bloom (HAB). These HABs cause disturbances in many aquatic ecosystems (e.g. harming fish, shellfish, and other aquatic organisms) through different mechanisms such as production of toxins or depletion of dissolved oxygen.[387] *Fischerella* and *Serratia* species are possibly negatively affected by bloom-forming phytoplankton. Thus, the production of allelochemicals by both genera could be a survival strategy to suppress organisms causing HABs. The increase in prodigiosin production of *Serratia* species due to exposure to ambigols that are secreted by *F. ambigua* could be a mutual relationship with the aim to avoid the negative impacts of HABs in their ecosystem. Future studies could investigate the bacterial interactions between *Serratia* and *Fischerella* species as well as their potential relationships to plants and bloom-forming phytoplankton as there are no reports regarding these topics. Valuable new information from such investigations could improve the potential application of *Serratia* and *Fischerella* species as biofertilizers in agriculture or as biocontrol agents against HABs.

5 Conclusion

The aim of this thesis was to identify and characterize new natural products from cyanobacteria for drug development purposes utilizing a chemistry-guided and two distinct bioassay-based screening approaches. The chemistry-guided screening approach utilized the software tool HaloSeeker to filter for ion signals of halogenated compounds in complex HRMS data sets from cyanobacteria, resulting in the isolation of new tjipanazoles from *F. ambigua*. Moreover, the detection of additional tjipanazole intermediates by HaloSeeker allowed us to revisit and substantiate the putative tjipanazole biosynthesis pathway. Based on these promising results, the use of this software for the isolation of new HNPs or the elucidation of their corresponding biosynthetic pathways can be recommended for future studies. Furthermore, a conducted structure-activity relationship study indicated the importance of various structural features of indolo[2,3-a]carbazoles for their capability to inhibit the breast cancer transporter ABCG2. These findings can be of valuable information for future studies investigating indolocarbazoles with ABCG2 inhibitory activity.

Bioactivity-based screening campaigns for immunomodulators and QS inhibitors from cyanobacteria resulted in the isolation of new hapalindoles and ambigols, respectively. The observed immunomodulating activity of the hapalindoles has not yet been described. Subsequent studies about toxicity and immunomodulatory effects are recommended to better understand the potential use of hapalindoles as immunomodulatory drugs. Although in this study, no QS inhibitors were identified from cyanobacteria, new ambigols with prodiginine-promoting activity were isolated from *F. ambigua*. In order to increase the probability of finding QS inhibitors in future studies, target-based assays that monitor the direct effect of samples on the QS system of a bacterium (e.g. mutant with *lacZ* fusion behind a QS gene) could be used alternatively to phenotypic assays. Since the Centers for Disease Control and Prevention has proclaimed beta-lactam resistant *Enterobacteriaceae* as an urgent threat to humans and *Serratia* is a member of this family, finding new antibiotics or alternative therapeutic drugs such as QS inhibitors should be subject of future investigations.

As seen in this study, phenotypic screenings have the advantage that unexpected though interesting effects can be observed. The increase in prodiginine production in *Serratia* species due to exposure to ambigols has not been reported yet. The mode-of-action for this activity could not unambiguously be identified using RNA-Seq. However, our results

indicate that **81** has a major impact on the translation machinery and the precursor supply of the prodiginine biosynthesis in S39006. Based on these results, future research might analyze the proteome and the precursor concentrations of S39006 treated with **81**. This study illustrated the ability of *Serratia* to sense molecules from *Fischerella* and change their phenotype accordingly, but also raises the questions if and in what relationship *Serratia* and *Fischerella* interact with each other. Subsequent studies could investigate the potential use of ambigols as a prodigiosin-promoting agent in the industrial production of prodigiosin or the combined use of *Fischerella* and *Serratia* cultures as biofertilizers in agriculture and as biocontrol agents against HABs.

All three screening campaigns resulted in the isolation and characterization of new bioactive natural products. The potential use of the isolated compounds as candidates for drug development or other applications remains to be investigated in future studies. The results presented here confirm that cyanobacteria represent a prolific source for new bioactive natural products and that chemistry-guided and bioactivity-based screening campaigns are suitable methods for their isolation.

6 Abbreviations

HPLC	high performance liquid chromatography
ABCG2	ATP-binding cassette super-family G member 2
ACN	acetonitrile
ADEQUATE	adequate double quantum transfer experiment
AHL	*N*-acyl-homoserine lactone
AI-2	autoinducer-2
AIP	autoinducing peptide
ATP	adenosine triphosphate
BGC	biosynthetic gene cluster
CDK	cyclin-dependent kinase
DAD	diode array detector
DEG	differential expressed gene
DMSO	dimethylsulfoxid
ELSD	evaporative light scattering detector
ESI	electrospray ionization
FAD	flavin adenine dinucleotide
FC	fold change
GO	gene ontology
HAB	harmful algal bloom
HMBC	heteronuclear multiple bond coherence
HNP	halogenated natural product
HPLC-MS	high performance liquid chromatography coupled to a mass spectrometer
HRESIMS	high resolution mass spectrometry with electrospray ionization
HRMS	high resolution mass spectrometry
$HRMS^2$	high resolution tandem mass spectrometry
HSQC	heteronuclear single quantum coherence
IC_{50}	half maximal inhibitory concentration
KEGG	Kyoto Encyclopedia of Genes and Genomes
m/*z*	mass-to-charge ratio
MAP	2-methyl-3-pentyl-1H-pyrrole
MBC	4-methoxy-2,2'-bipyrrole-5-carbaldehyde
MeOH	methanol
MS	mass spectrometry
NMR	nuclear magnetic resonance
NOESY	nuclear overhauser effect spectroscopy
NRPS	nonribosomale peptide synthetase
OD	optical density
PBDE	polybrominated diphenyl ethers
PKC	protein kinase C
PKS	polyketide synthase
ppm	parts per million
QS	quorum sensing
RLU	relative light units
RNA-Seq	RNA-sequencing
S39006	*Serratia* sp. ATCC 39006

References

(1) Harvey, A. L. Natural products in drug discovery. *Drug discovery today* 2008, *13*, 894–901.

(2) Newman, D. J.; Cragg, G. M. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J. Nat. Prod.* 2020, *83*, 770–803.

(3) Schopf, J. W.; Packer, B. M. Early Archean (3.3-billion to 3.5-billion-year-old) microfossils from Warrawoona Group, Australia. *Science* 1987, *237*, 70–73.

(4) Whitton, B. A. *Ecology of cyanobacteria II: their diversity in space and time*; Springer Science & Business Media, 2012.

(5) Shah, S. A. A.; Akhter, N.; Auckloo, B. N.; Khan, I.; Lu, Y.; Wang, K.; Wu, B.; Guo, Y.-W. Structural Diversity, Biological Properties and Applications of Natural Products from Cyanobacteria. A Review. *Mar. Drugs* 2017, *15*, 354.

(6) Welker, M.; Dohren, H. von. Cyanobacterial peptides - nature's own combinatorial biosynthesis. *FEMS Microbiol. Rev.* 2006, *30*, 530–563.

(7) Jones, A. C.; Gu, L.; Sorrels, C. M.; Sherman, D. H.; Gerwick, W. H. New tricks from ancient algae: natural products biosynthesis in marine cyanobacteria. *Curr. Opin. Chem. Biol.* 2009, *13*, 216–223.

(8) Shih, P. M.; Wu, D.; Latifi, A.; Axen, S. D.; Fewer, D. P.; Talla, E.; Calteau, A.; Cai, F.; Tandeau de Marsac, N.; Rippka, R.*; et al.* Improving the coverage of the cyanobacterial phylum using diversity-driven genome sequencing. *PNAS* 2013, *110*, 1053–1058.

(9) Demay, J.; Bernard, C.; Reinhardt, A.; Marie, B. Natural Products from Cyanobacteria: Focus on Beneficial Activities. *Mar. Drugs* 2019, *17*, 320.

(10) Bouaïcha, N.; Miles, C. O.; Beach, D. G.; Labidi, Z.; Djabri, A.; Benayache, N. Y.; Nguyen-Quang, T. Structural diversity, characterization and toxicology of microcystins. *Toxins* 2019, *11*, 714.

(11) Bhat, V.; Dave, A.; MacKay, J. A.; Rawal, V. H. The Chemistry of Hapalindoles, Fischerindoles, Ambiguines, and Welwitindolinones. *The Alkaloids: Chemistry and Biology* 2014, *73*, 65–160.

(12) Moore, R. E.; Cheuk, C.; Patterson, G. M. L. Hapalindoles: New alkaloids from the blue-green alga *Hapalosiphon fontinalis*. *J. Am. Chem. Soc.* 1984, *106*, 6456–6457.

(13) Smitka, T. A.; Bonjouklian, R.; Doolin, L.; Jones, N. D.; Deeter, J. B.; Yoshida, W. Y.; Prinsep, M. R.; Moore, R. E.; Patterson, G. M. L. Ambiguine isonitriles, fungicidal hapalindole-type alkaloids from three genera of blue-green algae belonging to the *Stigonemataceae*. *J. Org. Chem.* 1992, *57*, 857–861.

(14) Golakoti, T.; Ogino, J.; Heltzel, C. E.; Le Husebo, T.; Jensen, C. M.; Larsen, L. K.; Patterson, G. M. L.; Moore, R. E.; Mooberry, S. L.; Corbett, T. H.*; et al.* Structure determination, conformational analysis, chemical stability studies, and antitumor evaluation of the cryptophycins. Isolation of 18 new analogs from *Nostoc* sp. strain GSV 224. *J. Am. Chem. Soc.* 1995, *117*, 12030–12049.

(15) Trimurtulu, G.; Ohtani, I.; Patterson, G. M. L.; Moore, R. E.; Corbett, T. H.; Valeriote, F. A.; Demchik, L. Total Structures of Cryptophycins, Potent Antitumor Depsipeptides from the Blue-Green Alga *Nostoc* sp. Strain GSV 224. *J. Am. Chem. Soc.* 1994, *116*, 4729–4737.

(16) Martin, C.; Oberer, L.; Ino, T.; König, W. A.; Busch, M.; Weckesser, J. Cyanopeptolins, new depsipeptides from the cyanobacterium *Microcystis* sp. PCC 7806. *J. Antibiot.* 1993, *46*, 1550–1556.

(17) Jokela, J.; Oftedal, L.; Herfindal, L.; Permi, P.; Wahlsten, M.; Døskeland, S. O.; Sivonen, K. Anabaenolysins, novel cytolytic lipopeptides from benthic *Anabaena* cyanobacteria. *PLoS ONE* 2012, *7*, e41222.

(18) Marner, F.-J.; Moore, R. E.; Hirotsu, K.; Clardy, J. Majusculamides A and B, two epimeric lipodipeptides from *Lyngbya majuscula* Gomont. *J. Org. Chem.* 1977, *42*, 2815–2819.

(19) Ishibashi, M.; Moore, R. E.; Patterson, G. M. L.; Xu, C.; Clardy, J. Scytophycins, cytotoxic and antimycotic agents from the cyanophyte *Scytonema pseudohofmanni*. *J. Org. Chem.* 1986, *51*, 5300–5306.

(20) Teruya, T.; Sasaki, H.; Kitamura, K.; Nakayama, T.; Suenaga, K. Biselyngbyaside, a macrolide glycoside from the marine cyanobacterium *Lyngbya* sp. *Org. Lett.* 2009, *11*, 2421–2424.

(21) Jaki, B.; Orjala, J.; Heilmann, J.; Linden, A.; Vogler, B.; Sticher, O. Novel extracellular diterpenoids with biological activity from the cyanobacterium *Nostoc commune*. *J. Nat. Prod.* 2000, *63*, 339–343.

(22) Pérez Gutiérrez, R. M.; Martínez Flores, A.; Vargas Solís, R.; Carmona Jimenez, J. Two new antibacterial norabietane diterpenoids from cyanobacteria, *Microcoleous lacustris*. *J. Nat. Med.* 2008, *62*, 328–331.

(23) Hayashi, T.; Hayashi, K.; Maeda, M.; Kojima, I. Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. *J. Nat. Prod.* 1996, *59*, 83–87.

(24) Entzeroth, M.; Moore, R. E.; Niemczura, W. P.; Patterson, G. M. L.; Shoolery, J. N. O-Acetyl-O-butyryl-O-carbamoyl-O,O-dimethyl-.alpha.-cyclodextrins from the cyanophyte *Tolypothrix byssoidea*. *J. Org. Chem.* 1986, *51*, 5307–5310.

(25) Kwan, J. C.; Teplitski, M.; Gunasekera, S. P.; Paul, V. J.; Luesch, H. Isolation and biological evaluation of 8-epi-malyngamide C from the Floridian marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.* 2010, *73*, 463–466.

(26) Kwan, J. C.; Meickle, T.; Ladwa, D.; Teplitski, M.; Paul, V.; Luesch, H. Lyngbyoic acid, a "tagged" fatty acid from a marine cyanobacterium, disrupts quorum sensing in *Pseudomonas aeruginosa*. *Mol. BioSyst.* 2011, *7*, 1205–1216.

(27) Carmichael, W. W.; Boyer, G. L. Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. *Harmful Algae* 2016, *54*, 194–212.

(28) Niedermeyer, T. H. J.; Brönstrup, M. Natural product drug discovery from microalgae. In *Microalgal Biotechnology: Integration and Economy*, 1. Aufl.; Posten, C., Walter, C., Eds.; Walter de Gruyter GmbH Co.KG: s.l., 2012.

(29) Niedermeyer, T. H. J. Anti-infective Natural Products from Cyanobacteria. *Planta Med.* 2015, *81*, 1309–1325.

(30) Harrigan, G. G.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Park, P. U.; Biggs, J.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. Isolation, structure determination, and biological activity of dolastatin 12 and lyngbyastatin 1 from *Lyngbya majuscula*/*Schizothrix calcicola* cyanobacterial assemblages. *J. Nat. Prod.* 1998, *61*, 1221–1225.

(31) Harrigan, G. G.; Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Nagle, D. G.; Paul, V. J.; Mooberry, S. L.; Corbett, T. H.; Valeriote, F. A. Symplostatin 1: A dolastatin 10 analogue from the marine cyanobacterium *Symploca hydnoides*. *J. Nat. Prod.* 1998, *61*, 1075–1077.

(32) Fennell, B. J.; Carolan, S.; Pettit, G. R.; Bell, A. Effects of the antimitotic natural product dolastatin 10, and related peptides, on the human malarial parasite *Plasmodium falciparum*. *J. Antimicrob. Chemother.* 2003, *51*, 833–841.

(33) Bai, R.; Pettit, G. R.; Hamel, E. Structure-activity studies with chiral isomers and with segments of the antimitotic marine peptide dolastatin 10. *Biochem. Pharmacol.* 1990, *40*, 1859–1864.

(34) Simmons, T. L.; Andrianasolo, E.; McPhail, K.; Flatt, P.; Gerwick, W. H. Marine natural products as anticancer drugs. *Mol. Cancer Ther.* 2005, *4*, 333–342.

(35) Kobayashi, M.; Natsume, T.; Tamaoki, S.; Watanabe, J.; Asano, H.; Mikami, T.; Miyasaka, K.; Miyazaki, K.; Gondo, M.; Sakakibara, K.*; et al.* Antitumor activity of TZT-1027, a novel dolastatin 10 derivative. *Jpn. J. Cancer Res.* 1997, *88*, 316–327.

(36) Natsume, T.; Watanabe, J.-i.; Koh, Y.; Fujio, N.; Ohe, Y.; Horiuchi, T.; Saijo, N.; Nishio, K.; Kobayashi, M. Antitumor activity of TZT-1027 (Soblidotin) against vascular endothelial growth factor-secreting human lung cancer *in vivo*. *Cancer Sci.* 2003, *94*, 826–833.

(37) Newman, D. J. The "Utility" of Highly Toxic Marine-Sourced Compounds. *Mar. Drugs* 2019, *17*, 324.

(38) Minich, S. S. Brentuximab vedotin: a new age in the treatment of Hodgkin lymphoma and anaplastic large cell lymphoma. *Ann. Pharmacother.* 2012, *46*, 377–383.

(39) Raja, R.; Hemaiswarya, S.; Ganesan, V.; Carvalho, I. S. Recent developments in therapeutic applications of Cyanobacteria. *Crit. Rev. Microbiol.* 2016, *42*, 394–405.

(40) Shih, C.; Teicher, B. A. Cryptophycins: a novel class of potent antimitotic antitumor depsipeptides. *Curr. Pharm. Des.* 2001, *7*, 1259–1276.

(41) Dixit, R. B.; Suseela, M. R. Cyanobacteria: potential candidates for drug discovery. *Antonie van Leeuwenhoek* 2013, *103*, 947–961.

(42) Verma, V. A.; Pillow, T. H.; DePalatis, L.; Li, G.; Phillips, G. L.; Polson, A. G.; Raab, H. E.; Spencer, S.; Zheng, B. The cryptophycins as potent payloads for antibody drug conjugates. *Bioorg. Med. Chem. Lett.* 2015, *25*, 864–868.

(43) Lobo, K.; Donado, C.; Cornelissen, L.; Kim, J.; Ortiz, R.; Peake, R. W. A.; Kellogg, M.; Alexander, M. E.; Zurakowski, D.; Kurgansky, K. E.*; et al.* A Phase 1, Dose-escalation, Double-blind, Block-randomized, Controlled Trial of Safety and Efficacy of

Neosaxitoxin Alone and in Combination with 0.2% Bupivacaine, with and without Epinephrine, for Cutaneous Anesthesia. *Anesthesiology* 2015, *123*, 873–885.

(44) Rodriguez-Navarro, A. J.; Lagos, N.; Lagos, M.; Braghetto, I.; Csendes, A.; Hamilton, J.; Figueroa, C.; Truan, D.; Garcia, C.; Rojas, A.*; et al.* Neosaxitoxin as a local anesthetic: preliminary observations from a first human trial. *Anesthesiology* 2007, *106*, 339–345.

(45) Mihali, T. K.; Kellmann, R.; Neilan, B. a. Characterisation of the paralytic shellfish toxin biosynthesis gene clusters in *Anabaena circinalis* AWQC131C and *Aphanizomenon* sp. NH-5. *BMC Biochem.* 2009, *10*, 8.

(46) Zhang, D.; Hu, C.; Wang, G.; Li, D.; Li, G.; Liu, Y. Zebrafish neurotoxicity from aphantoxins--cyanobacterial paralytic shellfish poisons (PSPs) from *Aphanizomenon flos-aquae* DC-1. *Environ. Toxicol.* 2013, *28*, 239–254.

(47) Moustaka-Gouni, M.; Hiskia, A.; Genitsaris, S.; Katsiapi, M.; Manolidi, K.; Zervou, S.-K.; Christophoridis, C.; Triantis, T. M.; Kaloudis, T.; Orfanidis, S. First report of *Aphanizomenon favaloroi* occurrence in Europe associated with saxitoxins and a massive fish kill in Lake Vistonis, Greece. *Mar. Freshwater Res.* 2017, *68*, 793.

(48) Penzotti, J. L.; Lipkind, G.; Fozzard, H. A.; Dudley, S. C. Specific neosaxitoxin interactions with the Na+ channel outer vestibule determined by mutant cycle analysis. *Biophys. J.* 2001, *80*, 698–706.

(49) Thottumkara, A. P.; Parsons, W. H.; Du Bois, J. Saxitoxin. *Angew. Chem. Int. Ed. Engl.* 2014, *53*, 5760–5784.

(50) Rodriguez-Navarro, A. J.; Lagos, M.; Figueroa, C.; Garcia, C.; Recabal, P.; Silva, P.; Iglesias, V.; Lagos, N. Potentiation of local anesthetic activity of neosaxitoxin with bupivacaine or epinephrine: development of a long-acting pain blocker. *Neurotoxic. Res.* 2009, *16*, 408–415.

(51) Montero, M. C.; del Campo, M.; Bono, M.; Simon, M. V.; Guerrero, J.; Lagos, N. Neosaxitoxin Inhibits the Expression of Inflammation Markers of the M1 Phenotype in Macrophages. *Mar. Drugs* 2020, *18*, 283.

(52) Cabrita, M. T.; Vale, C.; Rauter, A. P. Halogenated Compounds from Marine Algae. *Mar. Drugs* 2010, *8*, 2301–2317.

(53) Kasanah, N.; Triyanto, T. Bioactivities of Halometabolites from Marine Actinobacteria. *Biomolecules* 2019, *9*, 225.

(54) Moore, R. E. Volatile compounds from marine algae. *Acc. Chem. Res.* 1977, *10*, 40–47.

(55) Takahashi, Y.; Suzuki, M.; Abe, T.; Masuda, M. Japonenynes, halogenated C15 acetogenins from *Laurencia japonensis*. *Phytochemistry* 1999, *50*, 799–803.

(56) Ji, N.-Y.; Li, X.-M.; Wang, B.-G. Sesquiterpenes and Other Metabolites from the Marine Red Alga *Laurencia composita* (*Rhodomelaceae*). *Helv. Chim. Acta* 2010, *93*, 2281–2286.

(57) Liu, M.; Hansen, P. E.; Lin, X. Bromophenols in marine algae and their bioactivities. *Mar. Drugs* 2011, *9*, 1273–1292.

(58) Woolner, V. H.; Jones, C. M.; Field, J. J.; Fadzilah, N. H.; Munkacsi, A. B.; Miller, J. H.; Keyzers, R. A.; Northcote, P. T. Polyhalogenated Indoles from the Red Alga *Rhodophyllis membranacea*: The First Isolation of Bromo-Chloro-Iodo Secondary Metabolites. *J. Nat. Prod.* 2016, *79*, 463–469.

(59) Jesus, A.; Correia-da-Silva, M.; Afonso, C.; Pinto, M.; Cidade, H. Isolation and Potential Biological Applications of Haloaryl Secondary Metabolites from Macroalgae. *Mar. Drugs* 2019, *17*, 73.

(60) Utkina, N. K.; Fedoreyev, S. A.; Ilyin, S. G.; Antipin, M. Y. 3,5-Dibromo-2-methoxybenzoic acid from sea sponge *Didiscus* sp. *Russ. Chem. Bull.* 1998, *47*, 2292–2294.

(61) Pham, N. B.; Butler, M. S.; Hooper, J. N.; Moni, R. W.; Quinn, R. J. Isolation of xestosterol esters of brominated acetylenic fatty acids from the marine sponge *xestospongia testudinaria*. *J. Nat. Prod.* 1999, *62*, 1439–1442.

(62) Kusama, T.; Tanaka, N.; Sakai, K.; Gonoi, T.; Fromont, J.; Kashiwada, Y.; Kobayashi, J. i. Agelamadins A and B, dimeric bromopyrrole alkaloids from a marine sponge *Agelas* sp. *Org. Lett.* 2014, *16*, 3916–3918.

(63) Sheu, J.-H.; Sung, P.-J.; Su, J.-H.; Liu, H.-Y.; Duh, C.-Y.; Chiang, M. Y. Briaexcavatolides A–J, new diterpenes from the gorgonian *Briareum excavatum*. *Tetrahedron* 1999, *55*, 14555–14564.

(64) Qureshi, A.; Faulkner, D. J. 3,6-Dibromoindole, A New Indole From the Palauan Ascidian *Distaplia regina*. *Nat. Prod. Lett.* 1999, *13*, 59–62.

(65) Rudi, A.; Evan, T.; Aknin, M.; Kashman, Y. Polycitone B and prepolycitrin A: two novel alkaloids from the marine ascidian *Polycitor africanus*. *J. Nat. Prod.* 2000, *63*, 832–833.

(66) Smitha, D.; Kumar, M. M. K.; Ramana, H.; Rao, D. V. Rubrolide R: a new furanone metabolite from the ascidian *Synoicum* of the Indian Ocean. *Nat. Prod. Res.* 2014, *28*, 12–17.

(67) Varns, J. L. The release of methyl chloride from potato tubers. *Am. Potato J.* 1982, *59*, 593–604.

(68) Gan, J.; Yates, S. R.; Ohr, H. D.; Sims, J. J. Production of methyl bromide by terrestrial higher plants. *Geophys. Res. Lett.* 1998, *25*, 3595–3598.

(69) Zhu, Y.; Yang, L.; Jia, Z. J. Novel highly oxygenated bisabolane sesquiterpenes from *Cremanthodium discoideum*. *J. Nat. Prod.* 1999, *62*, 1479–1483.

(70) Jensen, S. R.; Gotfredsen, C. H.; Harput, U. S.; Saracoglu, I. Chlorinated iridoid glucosides from *Veronica longifolia* and their antioxidant activity. *J. Nat. Prod.* 2010, *73*, 1593–1596.

(71) Wang, J.; Han, N.; Wang, Y.; Wang, Y.; Liu, Z.; Wang, Y.; Yin, J. Three alkaloids from *Reineckia carnea* herba and their antitussive and expectorant activities. *Nat. Prod. Res.* 2014, *28*, 1306–1309.

(72) Nakanishi, S.; Ando, K.; Kawamoto, I.; Yasuzawa, T.; Sano, H.; Kase, H. KS-504 compounds, novel inhibitors of Ca2+ and calmodulin-dependent cyclic nucleotide phosphodiesterase from *Mollisia ventosa*. *J. Antibiot.* 1989, *42*, 1775–1783.

(73) Silk, P.J.; Aubry, C.; Lonergan, G.C.; Macaulay, J.B. Chlorometabolite production by the ecologically important white rot fungus *Bjerkandera adusta*. *Chemosphere* 2001, *44*, 1603–1616.

(74) Jesus, A. E. de; Steyn, P. S.; van Heerden, F. R.; Vleggaar, R.; Wessels, P. L.; Hull, W. E. Tremorgenic mycotoxins from *Penicillium crustosum*: isolation of penitrems A–F and the structure elucidation and absolute configuration of penitrem A. *J. Chem. Soc., Perkin Trans. 1* 1983, 1847–1856.

(75) Field, J. A.; Verhagen, F. J.M.; Jong, E. de. Natural organohalogen production by basidiomycetes. *Trends Biotechnol.* 1995, *13*, 451–456.

(76) Ishibashi, M.; Shintani, A.; Toume, K.; Yamamoto, Y. Dehydrofuligoic Acid, a New Yellow Pigment Isolated from the Myxomycete *Fuligo septica f. flava*. *Heterocycles* 2010, *82*, 839.

(77) Chang, Y.-C.; Lu, C.-K.; Chiang, Y.-R.; Wang, G.-J.; Ju, Y.-M.; Kuo, Y.-H.; Lee, T.-H. Diterpene glycosides and polyketides from *Xylotumulus gibbisporus*. *J. Nat. Prod.* 2014, *77*, 751–757.

(78) Levine, D. P. Vancomycin: a history. *Clin. Infect. Dis.* 2006, *42 Suppl 1*, 5-12.

(79) Breinholt, J.; Gürtler, H.; Kjær, A.; Nielsen, S. E.; Olsen, C. E.; Ranta, K.; Rojo, T. Streptopyrrole: an Antimicrobial Metabolite from *Streptomyces armeniacus*. *Acta Chem. Scand.* 1998, *52*, 1040–1044.

(80) Fukuda, D. S.; Mynderse, J. S.; Baker, P. J.; Berry, D. M.; Boeck, L. D.; Yao, R. C.; Mertz, F. P.; Nakatsukasa, W. M.; Mabe, J.; Ott, J. A80915, a new antibiotic complex produced by *Streptomyces aculeolatus*. Discovery, taxonomy, fermentation, isolation, characterization, and antibacterial evaluation. *J. Antibiot.* 1990, *43*, 623–633.

(81) Hopp, D. C.; Rhea, J.; Jacobsen, D.; Romari, K.; Smith, C.; Rabenstein, J.; Irigoyen, M.; Clarke, M.; Francis, L.; Luche, M.*; et al.* Neopyrrolomycins with broad spectrum antibacterial activity. *J. Nat. Prod.* 2009, *72*, 276–279.

(82) Jansen, R.; Sood, S.; Huch, V.; Kunze, B.; Stadler, M.; Müller, R. Pyrronazols, metabolites from the myxobacteria *Nannocystis pusilla* and *N. exedens*, are unusual chlorinated pyrone-oxazole-pyrroles. *J. Nat. Prod.* 2014, *77*, 320–326.

(83) Li, D.; Sihamala, O.; Bhulaidok, S.; Shen, L. Changes in the organic compounds following sun drying of edible black ant (*Polyrhachis vicina Roger*). *Acta Aliment.* 2009, *38*, 493–501.

(84) Khalil, M. A. K.; Rasmussen, R. A.; French, J. R. J.; Holt, J. A. The influence of termites on atmospheric trace gases: CH 4 , CO 2 , CHCl 3 , N 2 O, CO, H 2 , and light hydrocarbons. *J. Geophys. Res.* 1990, *95*, 3619.

(85) Berger, R. S. Occurrence of 2,6-dichlorophenol in *Dermacentor albipictus* and *Haemaphysalis leporispalustris* (Acari: Ixodidae). *J. Med. Entomol.* 1983, *20*, 103.

(86) Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. Epibatidine: a novel (chloropyridyl)azabicycloheptane with potent analgesic activity from an Ecuadoran poison frog. *J. Am. Chem. Soc.* 1992, *114*, 3475–3478.

(87) Fitch, R. W.; Spande, T. F.; Garraffo, H. M.; Yeh, H. J. C.; Daly, J. W. Phantasmidine: an epibatidine congener from the ecuadorian poison frog *Epipedobates anthonyi*. *J. Nat. Prod.* 2010, *73*, 331–337.

(88) Gribble, G. W. A recent survey of naturally occurring organohalogen compounds. *Environ. Chem.* 2015, *12*, 396–405.

(89) Weiss, C.; Figueras, E.; Borbely, A. N.; Sewald, N. Cryptophycins: cytotoxic cyclodepsipeptides with potential for tumor targeting. *J. Pept. Sci.* 2017, *23*, 514–531.

(90) Ersmark, K.; Del Valle, J. R.; Hanessian, S. Chemistry and biology of the aeruginosin family of serine protease inhibitors. *Angew. Chem. Int. Ed. Engl.* 2008, *47*, 1202–1223.

(91) Murakami, M.; Ishida, K.; Okino, T.; Okita, Y.; Matsuda, H.; Yamaguchi, K. Aeruginosins 98-A and B, trypsin inhibitors from the blue-green alga *Microcystis aeruginosa* (NIES-98). *Tetrahedron Lett.* 1995, *36*, 2785–2788.

(92) Ishida, K.; Matsuda, H.; Murakami, M. Four new microginins, linear peptides from the cyanobacterium *Microcystis aeruginosa*. *Tetrahedron* 1998, *54*, 13475–13484.

(93) Matern, U.; Oberer, L.; Falchetto, R. A.; Erhard, M.; König, W. A.; Herdman, M.; Weckesser, J. Scyptolin A and B, cyclic depsipeptides from axenic cultures of *Scytonema hofmanni* PCC 7110. *Phytochemistry* 2001, *58*, 1087–1095.

(94) Luesch, H.; Yoshida, W. Y.; Moore, R. E.; Paul, V. J.; Mooberry, S. L. Isolation, structure determination, and biological activity of Lyngbyabellin A from the marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.* 2000, *63*, 611–615.

(95) Stratmann, K.; Moore, R. E.; Bonjouklian, R.; Deeter, J. B.; Patterson, G. M. L.; Shaffer, S.; Smith, C. D.; Smitka, T. A. Welwitindolinones, Unusual Alkaloids from the Blue-Green Algae *Hapalosiphon welwitschii* and *Westiella intricata*. Relationship to Fischerindoles and Hapalinodoles. *J. Am. Chem. Soc.* 1994, *116*, 9935–9942.

(96) Park, A.; Moore, R. E.; Patterson, G. M.L. Fischerindole L, a new isonitrile from the terrestrial blue-green alga *fischerella muscicola*. *Tetrahedron Lett.* 1992, *33*, 3257–3260.

(97) Falch, B. S.; Koenig, G. M.; Wright, A. D.; Sticher, O.; Ruegger, H.; Bernardinelli, G. Ambigol A and B: new biologically active polychlorinated aromatic compounds from the terrestrial blue-green alga *Fischerella ambigua*. *J. Org. Chem.* 1993, *58*, 6570–6575.

(98) Leão, P. N.; Nakamura, H.; Costa, M.; Pereira, A. R.; Martins, R.; Vasconcelos, V.; Gerwick, W. H.; Balskus, E. P. Biosynthesis-Assisted Structural Elucidation of the

Bartolosides, Chlorinated Aromatic Glycolipids from Cyanobacteria. *Angew. Chem., Int. Ed.* 2015, *54*, 11063–11067.

(99) Bui, H. T. N.; Jansen, R.; Pham, H. T. L.; Mundt, S. Carbamidocyclophanes A-E, chlorinated paracyclophanes with cytotoxic and antibiotic activity from the Vietnamese cyanobacterium *Nostoc* sp. *J. Nat. Prod.* 2007, *70*, 499–503.

(100) Cardellina, J. H.; Marner, F. J.; Moore, R. E. Malyngamide A, a novel chlorinated metabolite of the marine cyanophyte *Lyngbya majuscula*. *J. Am. Chem. Soc.* 1979, *101*, 240–242.

(101) Bonjouklian, R.; Smitka, T. A.; Doolin, L. E.; Molloy, R.M.; Debono, M.; Shaffer, S. A.; Moore, R. E.; Stewart, J. B.; Patterson, G. M.L. Tjipanazoles, new antifungal agents from the blue-green alga *Tolypothrix tjipanasensis*. *Tetrahedron* 1991, *47*, 7739–7750.

(102) Neumann, C. S.; Fujimori, D. G.; Walsh, C. T. Halogenation strategies in natural product biosynthesis. *Chem. Biol.* 2008, *15*, 99–109.

(103) Wagner, C.; El Omari, M.; König, G. M. Biohalogenation: nature's way to synthesize halogenated metabolites. *J. Nat. Prod.* 2009, *72*, 540–553.

(104) Jones, A. C.; Monroe, E. A.; Eisman, E. B.; Gerwick, L.; Sherman, D. H.; Gerwick, W. H. The unique mechanistic transformations involved in the biosynthesis of modular natural products from marine cyanobacteria. *Nat. Prod. Rep.* 2010, *27*, 1048.

(105) Frank, A.; Seel, C. J.; Groll, M.; Gulder, T. Characterization of a Cyanobacterial Haloperoxidase and Evaluation of its Biocatalytic Halogenation Potential. *ChemBioChem* 2016, *17*, 2028–2032.

(106) Warrington, R.; Watson, W.; Kim, H. L.; Antonetti, F. R. An introduction to immunology and immunopathology. *Allergy, Asthma, Clin. Immunol.* 2011, *7 Suppl 1*, S1.

(107) Carrillo, J. L. M.; Rodríguez, F. P. C.; Coronado, O. G.; García, M. A. M.; Cordero, J. F. C. Physiology and Pathology of Innate Immune Response Against Pathogens. *Physiol. Pathol. Immunol.*

(108) Bonilla, F. A.; Oettgen, H. C. Adaptive immunity. *J. Allergy Clin. Immunol.* 2010, *125*, S33-40.

(109) Melero, I.; Hervas-Stubbs, S.; Glennie, M.; Pardoll, D. M.; Chen, L. Immunostimulatory monoclonal antibodies for cancer therapy. *Nature reviews. Cancer* 2007, *7*, 95–106.

(110) Kovarik, J. From immunosuppression to immunomodulation: current principles and future strategies. *Pathobiology* 2013, *80*, 275–281.

(111) Portalès, P.; Clot, J. Immunostimulants revisited: focus on the pharmacology of Ribomunyl. *BioDrugs* 2006, *20*, 81–84.

(112) Fireman, M.; DiMartini, A. F.; Armstrong, S. C.; Cozza, K. L. Immunosuppressants. *Psychosomatics* 2004, *45*, 354–360.

(113) Nielsen, C. H.; Balachandran, P.; Christensen, O.; Pugh, N. D.; Tamta, H.; Sufka, K. J.; Wu, X.; Walsted, A.; Schjørring-Thyssen, M.; Enevold, C.*; et al.* Enhancement of natural killer cell activity in healthy subjects by Immulina®, a Spirulina extract enriched for Braun-type lipoproteins. *Planta Med.* 2010, *76*, 1802–1808.

(114) Effmert, U.; Mundt, S.; Teuscher, E. Untersuchungen zur immunmodulatorischen Wirkung von Extrakten aus Cyanobakterien. *Allerg. Immunol. (Leipzig)* 1991, *37*, 97–102.

(115) Furmaniak, M. A.; Misztak, A. E.; Franczuk, M. D.; Wilmotte, A.; Waleron, M.; Waleron, K. F. Edible Cyanobacterial Genus Arthrospira: Actual State of the Art in Cultivation Methods, Genetics, and Application in Medicine. *Front. Microbiol.* 2017, *8*, 2541.

(116) Koehn, F. E.; Longley, R. E.; Reed, J. K. Microcolins A and B, new immunosuppressive peptides from the blue-green alga *Lyngbya majuscula*. *J. Nat. Prod.* 1992, *55*, 613–619.

(117) Zurawell, R. W.; Chen, H.; Burke, J. M.; Prepas, E. E. Hepatotoxic cyanobacteria: a review of the biological importance of microcystins in freshwater environments. *J. Toxicol. Environ. Health B Crit. Rev.* 2005, *8*, 1–37.

(118) Chorus, I.; Bartram, J. *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring, and management*; E & FN Spon: London, New York, 1999.

(119) Corbel, S.; Mougin, C.; Bouaïcha, N. Cyanobacterial toxins: modes of actions, fate in aquatic and soil ecosystems, phytotoxicity and bioaccumulation in agricultural crops. *Chemosphere* 2014, *96*, 1–15.

(120) Adamovsky, O.; Moosova, Z.; Pekarova, M.; Basu, A.; Babica, P.; Svihalkova Sindlerova, L.; Kubala, L.; Blaha, L. Immunomodulatory Potency of Microcystin, an Important Water-Polluting Cyanobacterial Toxin. *Environ. Sci. Technol.* 2015, *49*, 12457–12464.

(121) Moosova, Z.; Hrouzek, P.; Kapuscik, A.; Blaha, L.; Adamovsky, O. Immunomodulatory effects of selected cyanobacterial peptides in vitro. *Toxicon* 2018, *149*, 20–25.

(122) Miura, G. A.; Robinson, N. A.; Lawrence, W. B.; Pace, J. G. Hepatotoxicity of microcystin-LR in fed and fasted rats. *Toxicon* 1991, *29*, 337–346.

(123) Zhao, S.; Li, G.; Chen, J. A proteomic analysis of prenatal transfer of microcystin-LR induced neurotoxicity in rat offspring. *J. Proteomics* 2015, *114*, 197–213.

(124) Zegura, B. An Overview of the Mechanisms of Microcystin-LR Genotoxicity and Potential Carcinogenicity. *Mini Rev. Med. Chem.* 2016, *16*, 1042–1062.

(125) Gaynes, R. The Discovery of Penicillin—New Insights After More Than 75 Years of Clinical Use. *Emerging Infect. Dis.* 2017, *23*, 849–853.

(126) Ventola, C. L. The Antibiotic Resistance Crisis: Part 1: Causes and Threats. *Pharm. Ther*. 2015, *40*, 277–283.

(127) Ribeiro da Cunha, B.; Fonseca, L. P.; Calado, C. R. C. Antibiotic Discovery: Where Have We Come from, Where Do We Go? *Antibiotics (Basel, Switz.)* 2019, *8*, 45.

(128) Loeffler, J. M.; Garbino, J.; Lew, D.; Harbarth, S.; Rohner, P. Antibiotic consumption, bacterial resistance and their correlation in a Swiss university hospital and its adult intensive care units. *Scand. J. Infect. Dis.* 2003, *35*, 843–850.

(129) Goossens, H.; Ferech, M.; Vander Stichele, R.; Elseviers, M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* 2005, *365*, 579–587.

(130) Auta, A.; Hadi, M. A.; Oga, E.; Adewuyi, E. O.; Abdu-Aguye, S. N.; Adeloye, D.; Strickland-Hodge, B.; Morgan, D. J. Global access to antibiotics without prescription in community pharmacies: A systematic review and meta-analysis. *J. Infect.* 2019, *78*, 8–18.

(131) Morgan, D. J.; Okeke, I. N.; Laxminarayan, R.; Perencevich, E. N.; Weisenberg, S. Non-prescription antimicrobial use worldwide: a systematic review. *Lancet Infect. Dis.* 2011, *11*, 692–701.

(132) Forman, R. F.; Marlowe, D. B.; McLellan, A. T. The Internet as a source of drugs of abuse. *Curr. Psychiatry Rep.* 2006, *8*, 377–382.

(133) Mainous, A. G.; Everett, C. J.; Post, R. E.; Diaz, V. A.; Hueston, W. J. Availability of Antibiotics for Purchase Without a Prescription on the Internet. *Ann. Fam. Med.* 2009, *7*, 431–435.

(134) Centres for Disease Control and Prevention. *Antibiotic resistance threats in the United States, 2019*, 2019.

(135) Coates, A.; Hu, Y.; Bax, R.; Page, C. The future challenges facing the development of new antimicrobial drugs. *Nat. Rev. Drug Discovery* 2002, *1*, 895–910.

(136) Projan, S. J.; Shlaes, D. M. Antibacterial drug discovery: is it all downhill from here? *Clin. Microbiol. Infect.* 2004, *10*, 18–22.

(137) Engebrecht, J.; Nealson, K.; Silverman, M. Bacterial bioluminescence: Isolation and genetic analysis of functions from *Vibrio fischeri*. *Cell* 1983, *32*, 773–781.

(138) Eberhard, A.; Burlingame, A. L.; Eberhard, C.; Kenyon, G. L.; Nealson, K. H.; Oppenheimer, N. J. Structural identification of autoinducer of *Photobacterium fischeri* Luciferase. *Biochemistry* 1981, *20*, 2444–2449.

(139) Cvitkovitch, D. G.; Li, Y.-H.; Ellen, R. P. Quorum sensing and biofilm formation in Streptococcal infections. *J. Clin. Invest.* 2003, *112*, 1626–1632.

(140) Davies, D. G.; Parsek, M. R.; Pearson, J. P.; Iglewski, B. H.; Costerton, J. W.; Greenberg, E. P. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 1998, *280*, 295–298.

(141) Lewenza, S.; Conway, B.; Greenberg, E. P.; Sokol, P. A. Quorum Sensing in *Burkholderia cepacia*: Identification of the LuxRI Homologs CepRI. *J. Bacteriol.* 1999, *181*, 748–756.

(142) Stintzi, A.; Evans, K.; Meyer, J. M.; Poole, K. Quorum-sensing and siderophore biosynthesis in *Pseudomonas aeruginosa*: *lasR*/*lasI* mutants exhibit reduced pyoverdine biosynthesis. *FEMS Microbiol. Lett.* 1998, *166*, 341–345.

(143) Grossman, A. D. Genetic networks controlling the initiation of sporulation and the development of genetic competence in *Bacillus subtilis*. *Annu. Rev. Genet.* 1995, *29*, 477–508.

(144) Perego, M. A peptide export-import control circuit modulating bacterial development regulates protein phosphatases of the phosphorelay. *Proc. Natl. Acad. Sci. U. S. A.* 1997, *94*, 8612–8617.

(145) Givskov, M. Control of exoenzyme production, motility and cell differentiation in *Serratia liquefaciens*. *FEMS Microbiol. Lett.* 1997, *148*, 115–122.

(146) Atkinson, S.; Throup, J. P.; Stewart, G. S.; Williams, P. A hierarchical quorum-sensing system in *Yersinia pseudotuberculosis* is involved in the regulation of motility and clumping. *Mol. Microbiol.* 1999, *33*, 1267–1277.

(147) Bainton, N. J.; Stead, P.; Chhabra, S. R.; Bycroft, B. W.; Salmond, G. P.; Stewart, G. S.; Williams, P. *N*-(3-oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. *Biochem. J.* 1992, *288 (Pt 3)*, 997–1004.

(148) Liu, X.; Bimerew, M.; Ma, Y.; Müller, H.; Ovadis, M.; Eberl, L.; Berg, G.; Chernin, L. Quorum-sensing signaling is required for production of the antibiotic pyrrolnitrin in a rhizospheric biocontrol strain of *Serratia plymuthica*. *FEMS Microbiol. Lett.* 2007, *270*, 299–305.

(149) Duerkop, B. A.; Varga, J.; Chandler, J. R.; Peterson, S. B.; Herman, J. P.; Churchill, M. E. A.; Parsek, M. R.; Nierman, W. C.; Greenberg, E. P. Quorum-sensing control of antibiotic synthesis in *Burkholderia thailandensis*. *J. Bacteriol.* 2009, *191*, 3909–3918.

(150) Novick, R. P.; Ross, H. F.; Projan, S. J.; Kornblum, J.; Kreiswirth, B.; Moghazeh, S. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. *Embo J.* 1993, *12*, 3967–3975.

(151) Pearson, J. P.; Feldman, M.; Iglewski, B. H.; Prince, A. *Pseudomonas aeruginosa* cell-to-cell signaling is required for virulence in a model of acute pulmonary infection. *Infect. Immun.* 2000, *68*, 4331–4334.

(152) Williams, P.; Winzer, K.; Chan, W. C.; Camara, M. Look who's talking: communication and quorum sensing in the bacterial world. *Philos. Trans. R. Soc., B* 2007, *362*, 1119–1134.

(153) Pesci, E. C.; Milbank, J. B.; Pearson, J. P.; McKnight, S.; Kende, A. S.; Greenberg, E. P.; Iglewski, B. H. Quinolone signaling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. U.S.A.* 1999, *96*, 11229–11234.

(154) Barber, C. E.; Tang, J. L.; Feng, J. X.; Pan, M. Q.; Wilson, T. J.; Slater, H.; Dow, J. M.; Williams, P.; Daniels, M. J. A novel regulatory system required for pathogenicity of *Xanthomonas campestris* is mediated by a small diffusible signal molecule. *Mol. Microbiol.* 1997, *24*, 555–566.

(155) Higgins, D. A.; Pomianek, M. E.; Kraml, C. M.; Taylor, R. K.; Semmelhack, M. F.; Bassler, B. L. The major *Vibrio cholerae* autoinducer and its role in virulence factor production. *Nature* 2007, *450*, 883–886.

(156) Flavier, A. B.; Clough, S. J.; Schell, M. A.; Denny, T. P. Identification of 3-hydroxypalmitic acid methyl ester as a novel autoregulator controlling virulence in *Ralstonia solanacearum*. *Mol. Microbiol.* 1997, *26*, 251–259.

(157) Surette, M. G.; Miller, M. B.; Bassler, B. L. Quorum sensing in *Escherichia coli, Salmonella typhimurium*, and *Vibrio harveyi*: a new family of genes responsible for autoinducer production. *Proc. Natl. Acad. Sci. U.S.A.* 1999, *96*, 1639–1644.

(158) Pereira, C. S.; Thompson, J. A.; Xavier, K. B. AI-2-mediated signalling in bacteria. *FEMS Microbiol. Rev.* 2013, *37*, 156–181.

(159) Haque, S.; Ahmad, F.; Dar, S. A.; Jawed, A.; Mandal, R. K.; Wahid, M.; Lohani, M.; Khan, S.; Singh, V.; Akhter, N. Developments in strategies for Quorum Sensing virulence factor inhibition to combat bacterial drug resistance. *Microb. Pathog.* 2018, *121*, 293–302.

(160) Gonzalez, J. E.; Keshavan, N. D. Messing with bacterial quorum sensing. *Microbiol. Mol. Biol. Rev.* 2006, *70*, 859–875.

(161) Sintim, H. O.; Al Smith, J.; Wang, J. X.; Nakayama, S.; Yan, L. Paradigm shift in discovering next-generation anti-infective agents: targeting quorum sensing, c-di-GMP signaling and biofilm formation in bacteria with small molecules. *Future Med. Chem.* 2010, *2*, 1005–1035.

(162) Rampioni, G.; Leoni, L.; Williams, P. The art of antibacterial warfare: Deception through interference with quorum sensing-mediated communication. *Bioorg. Chem.* 2014, *55*, 60–68.

(163) Reuter, K.; Steinbach, A.; Helms, V. Interfering with Bacterial Quorum Sensing. *Perspect. Med. Chem.* 2016, *8*, 1–15.

(164) Rasmussen, T. B.; Givskov, M. Quorum sensing inhibitors: a bargain of effects. *Microbiology* 2006, *152*, 895–904.

(165) Kwan, J. C.; Teplitski, M.; Gunasekera, S. P.; Paul, V. J.; Luesch, H. Isolation and biological evaluation of 8-epi-malyngamide C from the Floridian marine cyanobacterium *Lyngbya majuscula*. *J. Nat. Prod.* 2010, *73*, 463–466.

(166) Dobretsov, S.; Teplitski, M.; Alagely, A.; Gunasekera, S. P.; Paul, V. J. Malyngolide from the cyanobacterium *Lyngbya majuscula* interferes with quorum sensing circuitry. *Environ. Microbiol. Rep.* 2010, *2*, 739–744.

(167) Pearson, J. P.; Pesci, E. C.; Iglewski, B. H. Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J. Bacteriol.* 1997, *179*, 5756–5767.

(168) Dobretsov, S.; Teplitski, M.; Bayer, M.; Gunasekera, S.; Proksch, P.; Paul, V. J. Inhibition of marine biofouling by bacterial quorum sensing inhibitors. *Biofouling* 2011, *27*, 893–905.

(169) Montaser, R.; Paul, V. J.; Luesch, H. Modular strategies for structure and function employed by marine cyanobacteria: characterization and synthesis of pitinoic acids. *Org Lett* 2013, *15*, 4050–4053.

(170) Clark, B. R.; Engene, N.; Teasdale, M. E.; Rowley, D. C.; Matainaho, T.; Valeriote, F. A.; Gerwick, W. H. Natural products chemistry and taxonomy of the marine cyanobacterium *Blennothrix cantharidosmum*. *J. Nat. Prod.* 2008, *71*, 1530–1537.

(171) Choi, H.; Mascuch, S. J.; Villa, F. A.; Byrum, T.; Teasdale, M. E.; Smith, J. E.; Preskitt, L. B.; Rowley, D. C.; Gerwick, L.; Gerwick, W. H. Honaucins A-C, potent inhibitors of inflammation and bacterial quorum sensing: synthetic derivatives and structure-activity relationships. *Chemistry & biology* 2012, *19*, 589–598.

(172) Meyer, J. L.; Gunasekera, S. P.; Scott, R. M.; Paul, V. J.; Teplitski, M. Microbiome shifts and the inhibition of quorum sensing by Black Band Disease cyanobacteria. *ISME J.* 2016, *10*, 1204–1216.

(173) Sharif, D. I.; Gallon, J.; Smith, C. J.; Dudley, E. Quorum sensing in Cyanobacteria: N-octanoyl-homoserine lactone release and response, by the epilithic colonial cyanobacterium *Gloeothece* PCC6909. *ISME J.* 2008, *2*, 1171–1182.

(174) Gerdt, J. P.; Blackwell, H. E. Competition studies confirm two major barriers that can preclude the spread of resistance to quorum-sensing inhibitors in bacteria. *ACS Chem. Biol.* 2014, *9*, 2291–2299.

(175) Sudano Roccaro, A.; Blanco, A. R.; Giuliano, F.; Rusciano, D.; Enea, V. Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. *Antimicrob. Agents Chemother.* 2004, *48*, 1968–1973.

(176) Christensen, L. D.; van Gennip, M.; Jakobsen, T. H.; Alhede, M.; Hougen, H. P.; Høiby, N.; Bjarnsholt, T.; Givskov, M. Synergistic antibacterial efficacy of early combination treatment with tobramycin and quorum-sensing inhibitors against *Pseudomonas aeruginosa* in an intraperitoneal foreign-body infection mouse model. *J. Antimicrob. Chemother.* 2012, *67*, 1198–1206.

(177) Furiga, A.; Lajoie, B.; El Hage, S.; Baziard, G.; Roques, C. Impairment of *Pseudomonas aeruginosa* Biofilm Resistance to Antibiotics by Combining the Drugs with a New Quorum-Sensing Inhibitor. *Antimicrob. Agents Chemother.* 2015, *60*, 1676–1686.

(178) Bahari, S.; Zeighami, H.; Mirshahabi, H.; Roudashti, S.; Haghi, F. Inhibition of *Pseudomonas aeruginosa* quorum sensing by subinhibitory concentrations of curcumin with gentamicin and azithromycin. *J. Glob. Antimicrob. Resist.* 2017, *10*, 21–28.

(179) Walz, J. M.; Avelar, R. L.; Longtine, K. J.; Carter, K. L.; Mermel, L. A.; Heard, S. O. Anti-infective external coating of central venous catheters: a randomized, noninferiority trial comparing 5-fluorouracil with chlorhexidine/silver sulfadiazine in preventing catheter colonization. *Crit. Care Med.* 2010, *38*, 2095–2102.

(180) Smyth, A. R.; Cifelli, P. M.; Ortori, C. A.; Righetti, K.; Lewis, S.; Erskine, P.; Holland, E. D.; Givskov, M.; Williams, P.; Cámara, M.*; et al.* Garlic as an inhibitor of *Pseudomonas aeruginosa* quorum sensing in cystic fibrosis--a pilot randomized controlled trial. *Pediatr. Pulmonol* 2010, *45*, 356–362.

(181) van Delden, C.; Köhler, T.; Brunner-Ferber, F.; François, B.; Carlet, J.; Pechère, J.-C. Azithromycin to prevent *Pseudomonas aeruginosa* ventilator-associated pneumonia by inhibition of quorum sensing: a randomized controlled trial. *Intensive Care Med.* 2012, *38*, 1118–1125.

(182) Grimont, F., & Grimont, P. A., Ed. *The genus serratia*: *Vol. 6: Proteobacteria: Gamma Subclass*; Springer Science & Business Media, 2006.

(183) Jones, R. N. Microbial etiologies of hospital-acquired bacterial pneumonia and ventilator-associated bacterial pneumonia. *Clin. Infect. Dis.* 2010, *51*, S81–87.

(184) Blossom, D.; Noble-Wang, J.; Su, J.; Pur, S.; Chemaly, R.; Shams, A.; Jensen, B.; Pascoe, N.; Gullion, J.; Casey, E.*; et al.* Multistate outbreak of *Serratia marcescens* bloodstream infections caused by contamination of prefilled heparin and isotonic sodium chloride solution syringes. *Arch. Intern. Med.* 2009, *169*, 1705–1711.

(185) Us, E.; Kutlu, H. H.; Tekeli, A.; Ocal, D.; Cirpan, S.; Memikoglu, K. O. Wound and soft tissue infections of *Serratia marcescens* in patients receiving wound care: A health care-associated outbreak. *Am. J. Infect. Control* 2017, *45*, 443–447.

(186) Maki, D. G.; Hennekens, C. G.; Phillips, C. W.; Shaw, W. V.; Bennett, J. V. Nosocomial urinary tract infection with *Serratia marcescens*: an epidemiologic study. *J. Infect. Dis.* 1973, *128*, 579–587.

(187) Knowles, S.; Herra, C.; Devitt, E.; O'Brien, A.; Mulvihill, E.; McCann, S. R.; Browne, P.; Kennedy, M. J.; Keane, C. T. An outbreak of multiply resistant *Serratia marcescens*: the importance of persistent carriage. *Bone Marrow Transplant.* 2000, *25*, 873–877.

(188) Arakawa, Y.; Ike, Y.; Nagasawa, M.; Shibata, N.; Doi, Y.; Shibayama, K.; Yagi, T.; Kurata, T. Trends in antimicrobial-drug resistance in Japan. *Emerging Infect. Dis.* 2000, *6*, 572–575.

(189) van Houdt, R.; Givskov, M.; Michiels, C. W. Quorum sensing in *Serratia*. *FEMS Microbiol. Rev.* 2007, *31*, 407–424.

(190) Thomson, N. R.; Crow, M. A.; McGowan, S. J.; Cox, A.; Salmond, G. P. Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Mol. Microbiol.* 2000, *36*, 539–556.

(191) Eberl, L.; Winson, M. K.; Sternberg, C.; Stewart, G. S.; Christiansen, G.; Chhabra, S. R.; Bycroft, B.; Williams, P.; Molin, S.; Givskov, M. Involvement of *N*-acyl-L-homoserine lactone autoinducers in controlling the multicellular behaviour of *Serratia liquefaciens*. *Mol. Microbiol.* 1996, *20*, 127–136.

(192) Eberl, L.; Christiansen, G.; Molin, S.; Givskov, M. Differentiation of *Serratia liquefaciens* into swarm cells is controlled by the expression of the *flhD* master operon. *J. Bacteriol.* 1996, *178*, 554–559.

(193) Horng, Y.-T.; Deng, S.-C.; Daykin, M.; Soo, P.-C.; Wei, J.-R.; Luh, K.-T.; Ho, S.-W.; Swift, S.; Lai, H.-C.; Williams, P. The LuxR family protein SpnR functions as a

negative regulator of *N*-acylhomoserine lactone-dependent quorum sensing in *Serratia marcescens*. *Mol. Microbiol.* 2002, *45*, 1655–1671.

(194) Christensen, A. B.; Riedel, K.; Eberl, L.; Flodgaard, L. R.; Molin, S.; Gram, L.; Givskov, M. Quorum-sensing-directed protein expression in *Serratia proteamaculans* B5a. *Microbiology* 2003, *149*, 471–483.

(195) Labbate, M.; Queck, S. Y.; Koh, K. S.; Rice, S. A.; Givskov, M.; Kjelleberg, S. Quorum sensing-controlled biofilm development in *Serratia liquefaciens* MG1. *J. Bacteriol.* 2004, *186*, 692–698.

(196) Coulthurst, S. J.; Williamson, N. R.; Harris, A. K. P.; Spring, D. R.; Salmond, G. P. C. Metabolic and regulatory engineering of *Serratia marcescens*: mimicking phage-mediated horizontal acquisition of antibiotic biosynthesis and quorum-sensing capacities. *Microbiology* 2006, *152*, 1899–1911.

(197) Hall-Stoodley, L.; Costerton, J. W.; Stoodley, P. Bacterial biofilms: from the natural environment to infectious diseases. *Nat. Rev. Microbiol.* 2004, *2*, 95–108.

(198) Papp-Wallace, K. M.; Endimiani, A.; Taracila, M. A.; Bonomo, R. A. Carbapenems: past, present, and future. *Antimicrob. Agents Chemother.* 2011, *55*, 4943–4960.

(199) van Houdt, R.; Moons, P.; Aertsen, A.; Jansen, A.; Vanoirbeek, K.; Daykin, M.; Williams, P.; Michiels, C. W. Characterization of a *luxI*/*luxR*-type quorum sensing system and *N*-acyl-homoserine lactone-dependent regulation of exo-enzyme and antibacterial component production in *Serratia plymuthica* RVH1. *Res. Microbiol.* 2007, *158*, 150–158.

(200) Coulthurst, S. J.; Kurz, C. L.; Salmond, G. P. C. *luxS* mutants of *Serratia* defective in autoinducer-2-dependent ‘quorum sensing’show strain-dependent impacts on virulence and production of carbapenem and prodigiosin. *Microbiology* 2004, *150*, 1901–1910.

(201) Williamson, N. R.; Fineran, P. C.; Leeper, F. J.; Salmond, G. P. C. The biosynthesis and regulation of bacterial prodiginines. *Nat. Rev. Microbiol.* 2006, *4*, 887–899.

(202) Hu, D. X.; Withall, D. M.; Challis, G. L.; Thomson, R. J. Structure, Chemical Synthesis, and Biosynthesis of Prodiginine Natural Products. *Chem. Rev.* 2016, *116*, 7818–7853.

(203) Lapenda, J. C.; Silva, P. A.; Vicalvi, M. C.; Sena, K. X. F. R.; Nascimento, S. C. Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. *World J. Microbiol. Biotechnol.* 2015, *31*, 399–406.

(204) Herráez, R.; Mur, A.; Merlos, A.; Viñas, M.; Vinuesa, T. Using prodigiosin against some gram-positive and gram-negative bacteria and *Trypanosoma cruzi*. *J. Venom. Anim. Toxins Incl. Trop. Dis.* 2019, *25*, e20190001.

(205) John Jimtha, C.; Jishma, P.; Sreelekha, S.; Chithra, S.; Radhakrishnan, E. Antifungal properties of prodigiosin producing rhizospheric *Serratia* sp. *Rhizosphere* 2017, *3*, 105–108.

(206) Castro, A. J. Antimalarial activity of prodigiosin. *Nature* 1967, *213*, 903–904.

(207) Williamson, N. R.; Fineran, P. C.; Gristwood, T.; Chawrai, S. R.; Leeper, F. J.; Salmond, G. P. C. Anticancer and immunosuppressive properties of bacterial prodiginines. *Future Microbiol.* 2007, *2*, 605–618.

(208) Paik, P. K.; Rudin, C. M.; Brown, A.; Rizvi, N. A.; Takebe, N.; Travis, W.; James, L.; Ginsberg, M. S.; Juergens, R.; Markus, S.*; et al.* A phase I study of obatoclax mesylate, a Bcl-2 antagonist, plus topotecan in solid tumor malignancies. *Cancer Chemother. Pharmacol.* 2010, *66*, 1079–1085.

(209) Langer, C. J.; Albert, I.; Ross, H. J.; Kovacs, P.; Blakely, L. J.; Pajkos, G.; Somfay, A.; Zatloukal, P.; Kazarnowicz, A.; Moezi, M. M.*; et al.* Randomized phase II study of carboplatin and etoposide with or without obatoclax mesylate in extensive-stage small cell lung cancer. *Lung Cancer* 2014, *85*, 420–428.

(210) Arellano, M. L.; Borthakur, G.; Berger, M.; Luer, J.; Raza, A. A phase II, multicenter, open-label study of obatoclax mesylate in patients with previously untreated myelodysplastic syndromes with anemia or thrombocytopenia. *Clin. Lymphoma Myeloma Leuk.* 2014, *14*, 534–539.

(211) Oki, Y.; Copeland, A.; Hagemeister, F.; Fayad, L. E.; Fanale, M.; Romaguera, J.; Younes, A. Experience with obatoclax mesylate (GX15-070), a small molecule pan-Bcl-2 family antagonist in patients with relapsed or refractory classical Hodgkin lymphoma. *Blood* 2012, *119*, 2171–2172.

(212) Hood, D. W.; Heidstra, R.; Swoboda, U. K.; Hodgson, D. A. Molecular genetic analysis of proline and tryptophan biosynthesis in *Streptomyces coelicolor* A3(2):

interaction between primary and secondary metabolism — a review. *Gene* 1992, *115*, 5–12.

(213) Burger, S. R.; Bennett, J. W. Droplet enrichment factors of pigmented and nonpigmented *Serratia marcescens*: possible selective function for prodigiosin. *Appl. Environ. Microbiol.* 1985, *50*, 487–490.

(214) Matsuyama, T.; Murakami, T.; Fujita, M.; Fujita, S.; Yano, I. Extracellular Vesicle Formation and Biosurfactant Production by *Serratia marcescens*. *Microbiology* 1986, *132*, 865–875.

(215) Azambuja, P.; Feder, D.; Garcia, E. S. Isolation of *Serratia marcescens* in the midgut of *Rhodnius prolixus*: impact on the establishment of the parasite *Trypanosoma cruzi* in the vector. *Exp. Parasitol.* 2004, *107*, 89–96.

(216) Sakuraoka, R.; Suzuki, T.; Morohoshi, T. Distribution and Genetic Diversity of Genes Involved in Quorum Sensing and Prodigiosin Biosynthesis in the Complete Genome Sequences of *Serratia marcescens*. *Genome Biol. Evol.* 2019, *11*, 931–936.

(217) Harris, A. K. P.; Williamson, N. R.; Slater, H.; Cox, A.; Abbasi, S.; Foulds, I.; Simonsen, H. T.; Leeper, F. J.; Salmond, G. P. C. The *Serratia* gene cluster encoding biosynthesis of the red antibiotic, prodigiosin, shows species- and strain-dependent genome context variation. *Microbiology* 2004, *150*, 3547–3560.

(218) Williamson, N. R.; Simonsen, H. T.; Ahmed, R. A. A.; Goldet, G.; Slater, H.; Woodley, L.; Leeper, F. J.; Salmond, G. P. C. Biosynthesis of the red antibiotic, prodigiosin, in *Serratia*: identification of a novel 2-methyl-3-n-amyl-pyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in *Streptomyces*. *Mol. Microbiol.* 2005, *56*, 971–989.

(219) Couturier, M.; Bhalara, H. D.; Chawrai, S. R.; Monson, R.; Williamson, N. R.; Salmond, G. P. C.; Leeper, F. J. Substrate Flexibility of the Flavin-Dependent Dihydropyrrole Oxidases PigB and HapB Involved in Antibiotic Prodigiosin Biosynthesis. *ChemBioChem* 2020, *21*, 523–530.

(220) Thomas, M. G.; Burkart, M. D.; Walsh, C. T. Conversion of L-Proline to Pyrrolyl-2-Carboxyl-S-PCP during Undecylprodigiosin and Pyoluteorin Biosynthesis. *Chem. Biol.* 2002, *9*, 171–184.

(221) Fineran, P. C.; Slater, H.; Everson, L.; Hughes, K.; Salmond, G. P. C. Biosynthesis of tripyrrole and beta-lactam secondary metabolites in *Serratia*: integration of quorum

sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production. *Mol. Microbiol.* 2005, *56*, 1495–1517.

(222) Quintero-Yanes, A.; Lee, C. M.; Monson, R.; Salmond, G. The FloR master regulator controls flotation, virulence and antibiotic production in *Serratia* sp. ATCC 39006. *Environ. Microbiol.* 2020, *22*, 2921–2938.

(223) Wilf, N. M.; Salmond, G. P. C. The stationary phase sigma factor, RpoS, regulates the production of a carbapenem antibiotic, a bioactive prodigiosin and virulence in the enterobacterial pathogen *Serratia* sp. ATCC 39006. *Microbiology* 2012, *158*, 648–658.

(224) McNeil, M. B.; Clulow, J. S.; Wilf, N. M.; Salmond, G. P. C.; Fineran, P. C. SdhE is a conserved protein required for flavinylation of succinate dehydrogenase in bacteria. *J. Biol. Chem.* 2012, *287*, 18418–18428.

(225) Wilf, N. M.; Reid, A. J.; Ramsay, J. P.; Williamson, N. R.; Croucher, N. J.; Gatto, L.; Hester, S. S.; Goulding, D.; Barquist, L.; Lilley, K. S.*; et al.* RNA-seq reveals the RNA binding proteins, Hfq and RsmA, play various roles in virulence, antibiotic production and genomic flux in *Serratia* sp. ATCC 39006. *BMC genomics* 2013, *14*, 822.

(226) McNeil, M. B.; Iglesias-Cans, M. C.; Clulow, J. S.; Fineran, P. C. YgfX (CptA) is a multimeric membrane protein that interacts with the succinate dehydrogenase assembly factor SdhE (YgfY). *Microbiology* 2013, *159*, 1352–1365.

(227) Hampton, H. G.; McNeil, M. B.; Paterson, T. J.; Ney, B.; Williamson, N. R.; Easingwood, R. A.; Bostina, M.; Salmond, G. P. C.; Fineran, P. C. CRISPR-Cas gene-editing reveals RsmA and RsmC act through FlhDC to repress the SdhE flavinylation factor and control motility and prodigiosin production in *Serratia*. *Microbiology* 2016, *162*, 1047–1058.

(228) Gristwood, T.; Fineran, P. C.; Everson, L.; Salmond, G. P. C. PigZ, a TetR/AcrR family repressor, modulates secondary metabolism via the expression of a putative four-component resistance-nodulation-cell-division efflux pump, ZrpADBC, in *Serratia* sp. ATCC 39006. *Mol. Microbiol.* 2008, *69*, 418–435.

(229) Slater, H.; Crow, M.; Everson, L.; Salmond, G. P. C. Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and -independent pathways. *Mol. Microbiol.* 2003, *47*, 303–320.

(230) Gristwood, T.; Fineran, P. C.; Everson, L.; Williamson, N. R.; Salmond, G. P. The PhoBR two-component system regulates antibiotic biosynthesis in *Serratia* in response to phosphate. *BMC microbiology* 2009, *9*, 112.

(231) Quintero-Yanes, A.; Monson, R. E.; Salmond, G. P. C. Environmental potassium regulates bacterial flotation, antibiotic production and turgor pressure in *Serratia* through the TrkH transporter. *Environ. Microbiol.* 2019, *21*, 2499–2510.

(232) Williamson, N. R.; Fineran, P. C.; Ogawa, W.; Woodley, L. R.; Salmond, G. P. C. Integrated regulation involving quorum sensing, a two-component system, a GGDEF/EAL domain protein and a post-transcriptional regulator controls swarming and RhlA-dependent surfactant biosynthesis in *Serratia*. *Environ. Microbiol.* 2008, *10*, 1202–1217.

(233) Fineran, P. C.; Williamson, N. R.; Lilley, K. S.; Salmond, G. P. C. Virulence and prodigiosin antibiotic biosynthesis in *Serratia* are regulated pleiotropically by the GGDEF/EAL domain protein, PigX. *J. Bacteriol.* 2007, *189*, 7653–7662.

(234) Lee, C. M.; Monson, R. E.; Adams, R. M.; Salmond, G. P. C. The LacI-Family Transcription Factor, RbsR, Is a Pleiotropic Regulator of Motility, Virulence, Siderophore and Antibiotic Production, Gas Vesicle Morphogenesis and Flotation in *Serratia*. *Front. Microbiol.* 2017, *8*, 1678.

(235) Fineran, P. C.; Everson, L.; Slater, H.; Salmond, G. P. C. A GntR family transcriptional regulator (PigT) controls gluconate-mediated repression and defines a new, independent pathway for regulation of the tripyrrole antibiotic, prodigiosin, in *Serratia*. *Microbiology* 2005, *151*, 3833–3845.

(236) Léon, A.; Cariou, R.; Hutinet, S.; Hurel, J.; Guitton, Y.; Tixier, C.; Munschy, C.; Antignac, J.-P.; Dervilly-Pinel, G.; Le Bizec, B. HaloSeeker 1.0: A User-Friendly Software to Highlight Halogenated Chemicals in Nontargeted High-Resolution Mass Spectrometry Data Sets. *Anal. Chem.* 2019, *91*, 3500–3507.

(237) Knübel, G.; Larsen, L. K.; Moore, R. E.; Levine, I. A.; Patterson, G. M. Cytotoxic, antiviral indolocarbazoles from a blue-green alga belonging to the *Nostocaceae*. *J. Antibiot.* 1990, *43*, 1236–1239.

(238) Sánchez, C.; Méndez, C.; Salas, J. A. Indolocarbazole natural products: occurrence, biosynthesis, and biological activity. *Nat. Prod. Rep.* 2006, *23*, 1007–1045.

(239) Nettleton, D. E.; Doyle, T. W.; Krishnan, B.; Matsumoto, G. K.; Clardy, J. Isolation and structure of rebeccamycin - a new antitumor antibiotic from *nocardia aerocoligenes*. *Tetrahedron Lett.* 1985, *26*, 4011–4014.

(240) Omura, S.; Iwai, Y.; Hirano, A.; Nakagawa, A.; Awaya, J.; Tsuchya, H.; Takahashi, Y.; Masuma, R. A new alkaloid AM-2282 OF Streptomyces origin. Taxonomy, fermentation, isolation and preliminary characterization. *J. Antibiot.* 1977, *30*, 275–282.

(241) Kinnel, R. B.; Scheuer, P. J. 11-Hydroxystaurosporine: a highly cytotoxic, powerful protein kinase C inhibitor from a tunicate. *J. Org. Chem.* 1992, *57*, 6327–6329.

(242) Steglich, W. Slime moulds (Myxomycetes) as a source of new biologically active metabolites. *Pure Appl. Chem.* 1989, *61*, 281–288.

(243) Onaka, H.; Taniguchi, S.-i.; Igarashi, Y.; Furumai, T. Cloning of the staurosporine biosynthetic gene cluster from *Streptomyces* sp. TP-A0274 and its heterologous expression in *Streptomyces lividans*. *J. Antibiot.* 2002, *55*, 1063–1071.

(244) Salas, A. P.; Zhu, L.; Sánchez, C.; Braña, A. F.; Rohr, J.; Méndez, C.; Salas, J. A. Deciphering the late steps in the biosynthesis of the anti-tumour indolocarbazole staurosporine: sugar donor substrate flexibility of the StaG glycosyltransferase. *Mol. Microbiol.* 2005, *58*, 17–27.

(245) Sánchez, C.; Zhu, L.; Braña, A. F.; Salas, A. P.; Rohr, J.; Méndez, C.; Salas, J. A. Combinatorial biosynthesis of antitumor indolocarbazole compounds. *Proc. Natl. Acad. Sci. U. S. A.* 2005, *102*, 461–466.

(246) Altschul, S. F.; Gish, W.; Miller, W.; Myers, E. W.; Lipman, D. J. Basic local alignment search tool. *J. Mol. Biol.* 1990, *215*, 403–410.

(247) Du, Y.-L.; Williams, D. E.; Patrick, B. O.; Andersen, R. J.; Ryan, K. S. Reconstruction of cladoniamide biosynthesis reveals nonenzymatic routes to bisindole diversity. *ACS Chem. Biol.* 2014, *9*, 2748–2754.

(248) Onaka, H.; Taniguchi, S.-i.; Igarashi, Y.; Furumai, T. Characterization of the biosynthetic gene cluster of rebeccamycin from *Lechevalieria aerocolonigenes* ATCC 39243. *Biosci. Biotechnol. Biochem.* 2003, *67*, 127–138.

(249) Robey, R. W.; Shukla, S.; Steadman, K.; Obrzut, T.; Finley, E. M.; Ambudkar, S. V.; Bates, S. E. Inhibition of ABCG2-mediated transport by protein kinase inhibitors with

a bisindolylmaleimide or indolocarbazole structure. *Mol. Cancer Ther.* 2007, *6*, 1877–1885.

(250) Slater, M. J.; Cockerill, S.; Baxter, R.; Bonser, R. W.; Gohil, K.; Gowrie, C.; Robinson, J.E.; Littler, E.; Parry, N.; Randall, R.*; et al.* Indolocarbazoles: potent, selective inhibitors of human cytomegalovirus replication. *Bioorg. Med. Chem.* 1999, *7*, 1067–1074.

(251) Liu, R.; Zhu, T.; Li, D.; Gu, J.; Xia, W.; Fang, Y.; Liu, H.; Zhu, W.; Gu, Q. Two indolocarbazole alkaloids with apoptosis activity from a marine-derived actinomycete Z2039-2. *Arch. Pharmacal Res.* 2007, *30*, 270–274.

(252) Sanchez-Martinez, C.; Shih, C.; Faul, M. M.; Zhu, G.; Paal, M.; Somoza, C.; Li, T.; Kumrich, C. A.; Winneroski, L. L.; Xun, Z.*; et al.* Aryl[a]pyrrolo[3,4- c]carbazoles as selective cyclin D1-CDK4 inhibitors. *Bioorg. Med. Chem. Lett.* 2003, *13*, 3835–3839.

(253) Hirakawa, T.; Nasu, K.; Aoyagi, Y.; Takebayashi, K.; Narahara, H. Arcyriaflavin a, a cyclin D1-cyclin-dependent kinase4 inhibitor, induces apoptosis and inhibits proliferation of human endometriotic stromal cells: a potential therapeutic agent in endometriosis. *Reprod. Biol. Endocrinol.* 2017, *15*, 53.

(254) Jimenez, J. I.; Huber, U.; Moore, R. E.; Patterson, G. M. Oxidized welwitindolinones from terrestrial *fischerella* spp. *J. Nat. Prod.* 1999, *62*, 569–572.

(255) Moore, R. E.; Yang, X. Q. G.; Patterson, G. M. L. Fontonamide and anhydrohapaloxindole A, two new alkaloids from the blue-green alga *Hapalosiphon fontinalis*. *J. Org. Chem.* 1987, *52*, 3773–3777.

(256) Moore, R. E.; Yang, X.-q. G.; Patterson, G. M.L.; Bonjouklian, R.; Smitka, T. A. Hapalonamides and other oxidized hapalindoles from *Hapalosiphon fontinalis*. *Phytochemistry* 1989, *28*, 1565–1567.

(257) Kim, H.; Lantvit, D.; Hwang, C. H.; Kroll, D. J.; Swanson, S. M.; Franzblau, S. G.; Orjala, J. Indole alkaloids from two cultured cyanobacteria, *Westiellopsis* sp. and *Fischerella muscicola*. *Bioorg. Med. Chem.* 2012, *20*, 5290–5295.

(258) Bonjouklian, R.; Moore, R. E.; Patterson, G. M. L. Acid-catalyzed reactions of hapalindoles. *J. Org. Chem.* 1988, *53*, 5866–5870.

(259) Muratake, H.; Natsume, M. Synthetic studies of marine alkaloids hapalindoles. Part I Total synthesis of (±)-hapalindoles J and M. *Tetrahedron* 1990, *46*, 6331–6342.

(260) Aeri Park; Richard E. Moore; Gregory M.L Patterson. Fischerindole L, a new isonitrile from the terrestrial blue-green alga *Fischerella muscicola*. *Tetrahedron Lett.* 1992, *33*, 3257–3260.

(261) Chandra, A.; Johnston, J. N. Total synthesis of the chlorine-containing hapalindoles K, A, and G. *Angew. Chem. Int. Ed. Engl.* 2011, *50*, 7641–7644.

(262) Cagide, E.; Becher, P. G.; Louzao, M. C.; Espiña, B.; Vieytes, M. R.; Jüttner, F.; Botana, L. M. Hapalindoles from the cyanobacterium *fischerella*: potential sodium channel modulators. *Chem. Res. Toxicol.* 2014, *27*, 1696–1706.

(263) Falch, B. S.; König, G. M.; Wright, A. D.; Sticher, O.; Angerhofer, C. K.; Pezzuto, J. M.; Bachmann, H. Biological activities of cyanobacteria: evaluation of extracts and pure compounds. *Planta Med.* 1995, *61*, 321–328.

(264) Wright, A. D.; Papendorf, O.; Konig, G. M. Ambigol C and 2,4-dichlorobenzoic acid, natural products produced by the terrestrial cyanobacterium *Fischerella ambigua*. *J. Nat. Prod.* 2005, *68*, 459–461.

(265) Falch, B. S.; Koenig, G. M.; Wright, A. D.; Sticher, O.; Ruegger, H.; Bernardinelli, G. Ambigol A and B: new biologically active polychlorinated aromatic compounds from the terrestrial blue-green alga *Fischerella ambigua*. *J. Org. Chem.* 1993, *58*, 6570–6575.

(266) Darnerud, P. O.; Eriksen, G. S.; Jóhannesson, T.; Larsen, P. B.; Viluksela, M. Polybrominated diphenyl ethers: occurrence, dietary exposure, and toxicology. *Environ. Health Perspect.* 2001, *109 Suppl 1*, 49–68.

(267) Zhang, H.; Skildum, A.; Stromquist, E.; Rose-Hellekant, T.; Chang, L. C. Bioactive polybrominated diphenyl ethers from the marine sponge *Dysidea* sp. *J. Nat. Prod.* 2008, *71*, 262–264.

(268) Agarwal, V.; El Gamal, A. A.; Yamanaka, K.; Poth, D.; Kersten, R. D.; Schorn, M.; Allen, E. E.; Moore, B. S. Biosynthesis of polybrominated aromatic organic compounds by marine bacteria. *Nat. Chem. Biol.* 2014, *10*, 640–647.

(269) Agarwal, V.; Blanton, J. M.; Podell, S.; Taton, A.; Schorn, M. A.; Busch, J.; Lin, Z.; Schmidt, E. W.; Jensen, P. R.; Paul, V. J.*; et al.* Metagenomic discovery of polybrominated diphenyl ether biosynthesis by marine sponges. *Nat. Chem. Biol.* 2017, *13*, 537–543.

(270) Sharma, G. M.; Vig, B. Studies on the antimicrobial substances of sponges. VI. Structures of two antibacterial substances isolated from the marine sponge *Dysidea herbacea*. *Tetrahedron Lett.* 1972, *13*, 1715–1718.

(271) Carté, B.; Faulkner, D.J. Polybrominated diphenyl ethers from Dysidea herbacea, *Dysidea chlorea* and *Phyllospongia foliascens*. *Tetrahedron* 1981, *37*, 2335–2339.

(272) Fu, X.; Schmitz, F. J. New brominated diphenyl ether from an unidentified species of *Dysidea* sponge. 13C NMR data for some brominated diphenyl ethers. *J. Nat. Prod.* 1996, *59*, 1102–1103.

(273) Holmström; Kjelleberg. Marine *Pseudoalteromonas* species are associated with higher organisms and produce biologically active extracellular agents. *FEMS Microbiol. Ecol.* 1999, *30*, 285–293.

(274) Vetter, W.; Stoll, E.; Garson, M. J.; Fahey, S. J.; Gaus, C.; Müller, J. F. Sponge halogenated natural products found at parts-per-million levels in marine mammals. *Environ. Toxicol. Chem.* 2002, *21*, 2014–2019.

(275) Teuten, E. L.; Xu, L.; Reddy, C. M. Two abundant bioaccumulated halogenated compounds are natural products. *Science* 2005, *307*, 917–920.

(276) Wan, Y.; Choi, K.; Kim, S.; Ji, K.; Chang, H.; Wiseman, S.; Jones, P. D.; Khim, J. S.; Park, S.; Park, J.*; et al.* Hydroxylated polybrominated diphenyl ethers and bisphenol A in pregnant women and their matching fetuses: placental transfer and potential risks. *Environ. Sci. Technol.* 2010, *44*, 5233–5239.

(277) Wang, H.-S.; Chen, Z.-J.; Ho, K.-L.; Ge, L.-C.; Du, J.; Lam, M. H.-W.; Giesy, J. P.; Wong, M.-H.; Wong, C. K.-C. Hydroxylated and methoxylated polybrominated diphenyl ethers in blood plasma of humans in Hong Kong. *Environ. Int.* 2012, *47*, 66–72.

(278) Handayani, D.; Edrada, R. A.; Proksch, P.; Wray, V.; Witte, L.; van Soest, R. W.; Kunzmann, A.; Soedarsono. Four new bioactive polybrominated diphenyl ethers of the sponge *Dysidea herbacea* from *West Sumatra*, Indonesia. *J. Nat. Prod.* 1997, *60*, 1313–1316.

(279) Salvá, J.; Faulkner, D. J. A New Brominated Diphenyl Ether from a Philippine *Dysidea* Species. *J. Nat. Prod.* 1990, *53*, 757–760.

(280) Segraves, E. N.; Shah, R. R.; Segraves, N. L.; Johnson, T. A.; Whitman, S.; Sui, J. K.; Kenyon, V. A.; Cichewicz, R. H.; Crews, P.; Holman, T. R. Probing the activity

differences of simple and complex brominated aryl compounds against 15-soybean, 15-human, and 12-human lipoxygenase. *J. Med. Chem.* 2004, *47*, 4060–4065.

(281) Jones, R. D.; Jampani, H. B.; Newman, J. L.; Lee, A. S. Triclosan: a review of effectiveness and safety in health care settings. *Am. J. Infect. Control* 2000, *28*, 184–196.

(282) Bultel-Poncé; Debitus; Berge; Cerceau; Guyot. Metabolites from the sponge-associated bacterium *Micrococcus luteus*. *J. Mar. Biotechnol.* 1998, *6*, 233–236.

(283) Heath, R. J.; Rubin, J. R.; Holland, D. R.; Zhang, E.; Snow, M. E.; Rock, C. O. Mechanism of triclosan inhibition of bacterial fatty acid synthesis. *J. Biol. Chem.* 1999, *274*, 11110–11114.

(284) Liu, B.; Wang, Y.; Fillgrove, K. L.; Anderson, V. E. Triclosan inhibits enoyl-reductase of type I fatty acid synthase in vitro and is cytotoxic to MCF-7 and SKBr-3 breast cancer cells. *Cancer Chemother. Pharmacol.* 2002, *49*, 187–193.

(285) Honkisz, E.; Zieba-Przybylska, D.; Wojtowicz, A. K. The effect of triclosan on hormone secretion and viability of human choriocarcinoma JEG-3 cells. *Reprod. Toxicol* 2012, *34*, 385–392.

(286) Deepa, P. R.; Vandhana, S.; Jayanthi, U.; Krishnakumar, S. Therapeutic and toxicologic evaluation of anti-lipogenic agents in cancer cells compared with non-neoplastic cells. *Basic Clin. Pharmacol. Toxicol.* 2012, *110*, 494–503.

(287) Lu, S.; Archer, M. C. Fatty acid synthase is a potential molecular target for the chemoprevention of breast cancer. *Carcinogenesis* 2005, *26*, 153–157.

(288) Takahashi, A.; Agatsuma, T.; Matsuda, M.; Ohta, T.; Nunozawa, T.; Endo, T.; Nozoe, S. Russuphelin A, a new cytotoxic substance from the mushroom *Russula subnigricans* Hongo. *Chem. Pharm. Bull.* 1992, *40*, 3185–3188.

(289) Takahashi, A.; Agatsuma, T.; Ohta, T.; Nunozawa, T.; Endo, T.; Nozoe, S. Russuphelins B, C, D, E and F, new cytotoxic substances from the mushroom *Russula subnigricans* Hongo. *Chem. Pharm. Bull.* 1993, *41*, 1726–1729.

(290) Lee, I.-K.; Lee, J.-H.; Yun, B.-S. Polychlorinated compounds with PPAR-gamma agonistic effect from the medicinal fungus *Phellinus ribis*. *Bioorg. Med. Chem. Lett.* 2008, *18*, 4566–4568.

(291) Lee, P. T.; Wu, M. L.; Tsai, W. J.; Ger, J.; Deng, J. F.; Chung, H. M. Rhabdomyolysis: an unusual feature with mushroom poisoning. *Am. J. Kidney Dis.* 2001, *38*, E17.

(292) Matsuura, M.; Saikawa, Y.; Inui, K.; Nakae, K.; Igarashi, M.; Hashimoto, K.; Nakata, M. Identification of the toxic trigger in mushroom poisoning. *Nat. Chem. Biol.* 2009, *5*, 465–467.

(293) Cho, J. T.; Han, J. H. A Case of Mushroom Poisoning with *Russula subnigricans*: Development of Rhabdomyolysis, Acute Kidney Injury, Cardiogenic Shock, and Death. *J. Korean Med. Sci.* 2016, *31*, 1164–1167.

(294) Zhu, T.; Kim, S.-H.; Chen, C.-Y. A medicinal mushroom: *Phellinus linteus*. *Curr. Med. Chem.* 2008, *15*, 1330–1335.

(295) Zhang, B. B.; Moller, D. E. New approaches in the treatment of type 2 diabetes. *Curr. Opin. Chem. Biol.* 2000, *4*, 461–467.

(296) Murphy, G. J.; Holder, J. C. PPAR-γ agonists: therapeutic role in diabetes, inflammation and cancer. *Trends Pharmacol. Sci.* 2000, *21*, 469–474.

(297) Glombitza, K.-W.; Koch, M.; Eckhardt, G. Chlorierte phlorethole aus *Laminaria ochroleuca*. *Phytochemistry* 1977, *16*, 796–798.

(298) Glombitza, K. W.; Zieprath, G. Phlorotannins from the Brown Alga *Analipus japonicus*. *Planta Med.* 1989, *55*, 171–175.

(299) Sailler, B.; Glombitza, K.-W. Halogenated phlorethols and fucophlorethols from the brown alga *Cystophora retroflexa*. *Nat. Toxins* 1999, *7*, 57–62.

(300) Glombitza; Schmidt. Nonhalogenated and halogenated phlorotannins from the brown alga *carpophyllum angustifolium*. *J. Nat. Prod.* 1999, *62*, 1238–1240.

(301) Nwosu, F.; Morris, J.; Lund, V. A.; Stewart, D.; Ross, H. A.; McDougall, G. J. Anti-proliferative and potential anti-diabetic effects of phenolic-rich extracts from edible marine algae. *Food Chem.* 2011, *126*, 1006–1012.

(302) Kim, S. M.; Kang, K.; Jeon, J.-S.; Jho, E. H.; Kim, C. Y.; Nho, C. W.; Um, B.-H. Isolation of phlorotannins from *Eisenia bicyclis* and their hepatoprotective effect against oxidative stress induced by tert-butyl hyperoxide. *Appl. Biochem. Biotechnol.* 2011, *165*, 1296–1307.

(303) Kang, M.-C.; Cha, S. H.; Wijesinghe, W. A. J. P.; Kang, S.-M.; Lee, S.-H.; Kim, E.-A.; Song, C. B.; Jeon, Y.-J. Protective effect of marine algae phlorotannins against AAPH-induced oxidative stress in zebrafish embryo. *Food Chem.* 2013, *138*, 950–955.

(304) Kim, A.-R.; Shin, T.-S.; Lee, M.-S.; Park, J.-Y.; Park, K.-E.; Yoon, N.-Y.; Kim, J.-S.; Choi, J.-S.; Jang, B.-C.; Byun, D.-S.*; et al.* Isolation and identification of

phlorotannins from *Ecklonia stolonifera* with antioxidant and anti-inflammatory properties. *J. Agric. Food Chem.* 2009, *57*, 3483–3489.

(305) Eom, S.-H.; Kim, D.-H.; Lee, S.-H.; Yoon, N.-Y.; Kim, J. H.; Kim, T. H.; Chung, Y.-H.; Kim, S.-B.; Kim, Y.-M.; Kim, H.-W.*; et al. In vitro* antibacterial activity and synergistic antibiotic effects of phlorotannins isolated from *Eisenia bicyclis* against methicillin-resistant *Staphylococcus aureus*. *Phytother. Res.* 2013, *27*, 1260–1264.

(306) Ryu, B.; Li, Y.; Qian, Z.-J.; Kim, M.-M.; Kim, S.-K. Differentiation of human osteosarcoma cells by isolated phlorotannins is subtly linked to COX-2, iNOS, MMPs, and MAPK signaling: implication for chronic articular disease. *Chem. Biol. Interact.* 2009, *179*, 192–201.

(307) Yoon, N. Y.; Eom, T.-K.; Kim, M.-M.; Kim, S.-K. Inhibitory effect of phlorotannins isolated from *Ecklonia cava* on mushroom tyrosinase activity and melanin formation in mouse B16F10 melanoma cells. *J. Agric. Food Chem.* 2009, *57*, 4124–4129.

(308) Ścieszka, S.; Klewicka, E. Algae in food: a general review. *Crit. Rev. Food Sci. Nutr.* 2019, *59*, 3538–3547.

(309) Thomas, N. V.; Kim, S.-K. Potential pharmacological applications of polyphenolic derivatives from marine brown algae. *Environ. Toxicol. Pharmacol.* 2011, *32*, 325–335.

(310) Jahan, A.; Ahmad, I. Z.; Fatima, N.; Ansari, V. A.; Akhtar, J. Algal bioactive compounds in the cosmeceutical industry: a review. *Phycologia* 2017, *56*, 410–422.

(311) Terada, H. Uncouplers of oxidative phosphorylation. *Environ. Health Perspect.* 1990, *87*, 213–218.

(312) Wallace, K. B.; Starkov, A. A. Mitochondrial targets of drug toxicity. *Annu. Rev. Pharmacol. Toxicol.* 2000, *40*, 353–388.

(313) Basheer, M. M.; Custodio, R.; Volpe, P. L. O.; Rittner, R. An investigation of chlorophenol proton affinities and their influence on the biological activity of microorganisms. *J. Phys. Chem. A* 2006, *110*, 2021–2026.

(314) Ajao, C.; Andersson, M. A.; Teplova, V. V.; Nagy, S.; Gahmberg, C. G.; Andersson, L. C.; Hautaniemi, M.; Kakasi, B.; Roivainen, M.; Salkinoja-Salonen, M. Mitochondrial toxicity of triclosan on mammalian cells. *Toxicol. Rep.* 2015, *2*, 624–637.

(315) McLaughlin, S. G.; Dilger, J. P. Transport of protons across membranes by weak acids. *Physiol. Rev.* 1980, *60*, 825–863.

(316) Jensen, J. Chlorophenols in the terrestrial environment. *Rev. Environ. Contam. Toxicol.* 1996, *146*, 25–51.

(317) Legradi, J.; Dahlberg, A.-K.; Cenijn, P.; Marsh, G.; Asplund, L.; Bergman, Å.; Legler, J. Disruption of oxidative phosphorylation (OXPHOS) by hydroxylated polybrominated diphenyl ethers (OH-PBDEs) present in the marine environment. *Environ. Sci. Technol.* 2014, *48*, 14703–14711.

(318) Penttinen, O.-P. Chlorophenols in aquatic environments: structure-activity correlations. *Ann. Zool. Fenn.* 1995, 287–294.

(319) Garrity, L. F.; Ordal, G. W. Chemotaxis in *Bacillus subtilis*: How bacteria monitor environmental signals. *Pharmacol. Ther.* 1995, *68*, 87–104.

(320) Nicholas, R. A.; Ordal, G. W. Inhibition of bacterial transport by uncouplers of oxidative phosphorylation. Effects of pentachlorophenol and analogues in *Bacillus subtilis*. *Biochem. J.* 1978, *176*, 639–647.

(321) Brummett, T. B.; Ordal, G. W. Inhibition of amino acid transport in *Bacillus subtilis* by uncouplers of oxidative phosphorylation. *Arch. Biochem. Biophys.* 1977, *178*, 368–372.

(322) van den Broek, P. J.A.; van Steveninck, J. The influence of uncouplers on proton-sugar symport in *Saccharomyces fragilis*. *Biochim. Biophys. Acta, Bioenerg.* 1983, *722*, 102–106.

(323) Ehrlich, W.; Mangir, M.; Lochmann, E.-R. The effect of pentachlorophenol and its metabolite tetrachlorohydroquinone on RNA, protein, and ribosome synthesis in saccharomyces cells. *Ecotoxicol. Environ. Saf.* 1987, *13*, 7–12.

(324) Yadav, V.; Shitiz, K.; Pandey, R.; Yadav, J. Chlorophenol stress affects aromatic amino acid biosynthesis-a genome-wide study. *Yeast* 2011, *28*, 81–91.

(325) Legradi, J.; van Pomeren, M.; Dahlberg, A.-K.; Legler, J. Effects of Hydroxylated Polybrominated Diphenyl Ethers in Developing Zebrafish Are Indicative of Disruption of Oxidative Phosphorylation. *Int. J. Mol. Sci.* 2017, *18*, 970.

(326) van Boxtel, A. L.; Kamstra, J. H.; Cenijn, P. H.; Pieterse, B.; Wagner, J. M.; Antink, M.; Krab, K.; van der Burg, B.; Marsh, G.; Brouwer, A.*; et al.* Microarray analysis reveals a mechanism of phenolic polybrominated diphenylether toxicity in zebrafish. *Environ. Sci. Technol.* 2008, *42*, 1773–1779.

(327) Khalil, A.; Cevik, S. E.; Hung, S.; Kolla, S.; Roy, M. A.; Suvorov, A. Developmental Exposure to 2,2',4,4'-Tetrabromodiphenyl Ether Permanently Alters Blood-Liver Balance of Lipids in Male Mice. *Front. Endocrinol.* 2018, *9*, 548.

(328) Suvorov, A.; Takser, L. Delayed response in the rat frontal lobe transcriptome to perinatal exposure to the flame retardant BDE-47. *J. Appl. Toxicol.* 2011, *31*, 477–483.

(329) Khalil, A.; Parker, M.; Mpanga, R.; Cevik, S. E.; Thorburn, C.; Suvorov, A. Developmental Exposure to 2,2',4,4'-Tetrabromodiphenyl Ether Induces Long-Lasting Changes in Liver Metabolism in Male Mice. *J. Endocr. Soc.* 2017, *1*, 323–344.

(330) Weatherly, L. M.; Shim, J.; Hashmi, H. N.; Kennedy, R. H.; Hess, S. T.; Gosse, J. A. Antimicrobial agent triclosan is a proton ionophore uncoupler of mitochondria in living rat and human mast cells and in primary human keratinocytes. *J. Appl. Toxicol.* 2016, *36*, 777–789.

(331) Shim, J.; Weatherly, L. M.; Luc, R. H.; Dorman, M. T.; Neilson, A.; Ng, R.; Kim, C. H.; Millard, P. J.; Gosse, J. A. Triclosan is a mitochondrial uncoupler in live zebrafish. *J. Appl. Toxicol.* 2016, *36*, 1662–1667.

(332) Teplova, V. V.; Belosludtsev, K. N.; Kruglov, A. G. Mechanism of triclosan toxicity: Mitochondrial dysfunction including complex II inhibition, superoxide release and uncoupling of oxidative phosphorylation. *Toxicol. Lett.* 2017, *275*, 108–117.

(333) Popova, L. B.; Nosikova, E. S.; Kotova, E. A.; Tarasova, E. O.; Nazarov, P. A.; Khailova, L. S.; Balezina, O. P.; Antonenko, Y. N. Protonophoric action of triclosan causes calcium efflux from mitochondria, plasma membrane depolarization and bursts of miniature end-plate potentials. *Biochim. Biophys. Acta, Biomembr.* 2018, *1860*, 1000–1007.

(334) Bailey, A. M.; Constantinidou, C.; Ivens, A.; Garvey, M. I.; Webber, M. a.; Coldham, N.; Hobman, J. L.; Wain, J.; Woodward, M. J.; Piddock, L. J. V. Exposure of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium to triclosan induces a species-specific response, including drug detoxification. *J. Antimicrob. Chemother.* 2009, *64*, 973–985.

(335) Haddix, P. L.; Shanks, R. M. Q. Prodigiosin pigment of *Serratia marcescens* is associated with increased biomass production. *Arch. Microbiol.* 2018, *200*, 989–999.

(336) Haddix, P. L.; Shanks, R. M. Q. Production of prodigiosin pigment by Serratia *marcescens* is negatively associated with cellular ATP levels during high-rate, low-cell-density growth. *Can. J. Microbiol.* 2020, *66*, 243–255.

(337) Alihosseini, F.; Ju, K.-S.; Lango, J.; Hammock, B. D.; Sun, G. Antibacterial colorants: characterization of prodiginines and their applications on textile materials. *Biotechnol. Progr.* 2008, *24*, 742–747.

(338) Ahmad, W. A., Ed. *Application of bacterial pigments as colorant: The Malaysian perspective*; SpringerBriefs in Molecular Science; Springer: Heidelberg, New York, 2012.

(339) Jissa, G. K.; Ansu, J.; Philip, K.; Elyas, K. K.; M, C. Marine bacterial prodigiosin as dye for rubber latex, polymethyl methacrylate sheets and paper. *Afr. J. Biotechnol.* 2013, *12*, 2266–2269.

(340) Mehta, M.; Shah, G. Extraction of pigment from *Serratia marcescens* and its application in candle industry. *Adva. Appl. Rese.* 2015, *7*, 144–146.

(341) Ryazantseva, I.; Andreyeva, I. Application of prodigiosin as a colorant for polyolefines. *Adv. Biol. Chem.* 2014, *04*, 20–25.

(342) Giri, A. V.; Anandkumar, N.; Muthukumaran, G.; Pennathur, G. A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil. *BMC Microbiol.* 2004, *4*, 11.

(343) Elkenawy, N. M.; Yassin, A. S.; Elhifnawy, H. N.; Amin, M. A. Optimization of prodigiosin production by *Serratia marcescens* using crude glycerol and enhancing production using gamma radiation. *Biotechnol. Rep.* 2017, *14*, 47–53.

(344) Lin, C.; Jia, X.; Fang, Y.; Chen, L.; Zhang, H.; Lin, R.; Chen, J. Enhanced production of prodigiosin by *Serratia marcescens* FZSF02 in the form of pigment pellets. *Electron. J. Biotechnol.* 2019, *40*, 58–64.

(345) Wang, S.-L.; Wang, C.-Y.; Yen, Y.-H.; Liang, T.-W.; Chen, S.-Y.; Chen, C.-H. Enhanced production of insecticidal prodigiosin from *Serratia marcescens* TKU011 in media containing squid pen. *Process Biochem.* 2012, *47*, 1684–1690.

(346) Solé, M.; Rius, N.; Francia, A.; Lorén, J. G. The effect of pH on prodigiosin production by non-proliferating cells of *Serratia marcescens*. *Lett. Appl. Microbiol.* 1994, *19*, 341–344.

(347) Kurbanoglu, E. B.; Ozdal, M.; Ozdal, O. G.; Algur, O. F. Enhanced production of prodigiosin by *Serratia marcescens* MO-1 using ram horn peptone. *Braz. J. Microbiol.* 2015, *46*, 631–637.

(348) Sun, Y.; Wang, L.; Pan, X.; Osire, T.; Fang, H.; Zhang, H.; Yang, S.-T.; Yang, T.; Rao, Z. Improved Prodigiosin Production by Relieving CpxR Temperature-Sensitive Inhibition. *Front. Bioeng. Biotechnol.* 2020, *8*, 344.

(349) Amarouche-Yala, S.; Benouadah, A.; El Ouahab Bentabet, A.; López-García, P. Morphological and phylogenetic diversity of thermophilic cyanobacteria in Algerian hot springs. *Extremophiles* 2014, *18*, 1035–1047.

(350) Hirose, Y.; Fujisawa, T.; Ohtsubo, Y.; Katayama, M.; Misawa, N.; Wakazuki, S.; Shimura, Y.; Nakamura, Y.; Kawachi, M.; Yoshikawa, H.*; et al.* Complete genome sequence of cyanobacterium *Fischerella* sp. NIES-3754, providing thermoresistant optogenetic tools. *J. Biotechnol.* 2016, *220*, 45–46.

(351) Zakeri, F.; Noghabi, K. A.; Sadeghizadeh, M.; Kardan, M. R.; Masoomi, F.; Farshidpour, M. R.; Atarilar, A. *Serratia* sp. ZF03: an efficient radium biosorbent isolated from hot-spring waters in high background radiation areas. *Bioresour. Technol.* 2010, *101*, 9163–9170.

(352) Fiore, M. F.; Genuário, D. B.; da Silva, C. S. P.; Shishido, T. K.; Moraes, L. A. B.; Cantúsio Neto, R.; Silva-Stenico, M. E. Microcystin production by a freshwater spring cyanobacterium of the genus *Fischerella*. *Toxicon* 2009, *53*, 754–761.

(353) Khan, Z. U.; Tahmida Begum, Z. N.; Mandal, R.; Hossain, M. Z. Cyanobacteria in rice soils. *World J. Microbiol. Biotechnol.* 1994, *10*, 296–298.

(354) Ghasemi, Y.; Yazdi, M. T.; Shafiee, A.; Amini, M.; Shokravi, S.; Zarrini, G. Parsiguine, A Novel Antimicrobial Substance from *Fischerella ambigua*. *Pharm. Biol.* 2004, *42*, 318–322.

(355) Soltani, N.; Siahbalaie, R.; Shokravi, S. A New Description of *Fischerella Ambigua* (Näg.) Gom. - a Multidisciplinary Approach. *Int. J. Algae* 2010, *12*, 19–36.

(356) Tiwari, B.; Chakraborty, S.; Srivastava, A. K.; Mishra, A. K. Biodegradation and rapid removal of methyl parathion by the paddy field cyanobacterium *Fischerella* sp. *Algal Res.* 2017, *25*, 285–296.

(357) Rosales, A. M.; Vantomme, R.; Swings, J.; Ley, J.; Mew, T. W. Identification of some Bacteria from Paddy Antagonistic to Several Rice Fungal Pathogens. *J. Phytopathol.* 1993, *138*, 189–208.

(358) Gyaneshwar, P.; James, E. K.; Mathan, N.; Reddy, P. M.; Reinhold-Hurek, B.; Ladha, J. K. Endophytic colonization of rice by a diazotrophic strain of *Serratia marcescens*. *J. Bacteriol.* 2001, *183*, 2634–2645.

(359) Tripathi, A. K.; Verma, S. C.; Ron, E. Z. Molecular characterization of a salt-tolerant bacterial community in the rice rhizosphere. *Res. Microbiol.* 2002, *153*, 579–584.

(360) Zhang, C.-W.; Zhang, J.; Zhao, J.-J.; Zhao, X.; Zhao, D.-F.; Yin, H.-Q.; Zhang, X.-X. *Serratia oryzae* sp. nov., isolated from rice stems. *Int. J. Syst. Evol. Microbiol.* 2017, *67*, 2928–2933.

(361) Yang, F.; Wei, H. Y.; Li, X. Q.; Li, Y. H.; Li, X. B.; Yin, L. H.; Pu, Y. P. Isolation and characterization of an algicidal bacterium indigenous to lake Taihu with a red pigment able to lyse *microcystis aeruginosa*. *Biomed. Environ. Sci.* 2013, *26*, 148–154.

(362) Hugouvieux-Cotte-Pattat, N.; Jacot-des-Combes, C.; Briolay, J. Genomic characterization of a pectinolytic isolate of *Serratia oryzae* isolated from lake water. *J. Genomics* 2019, *7*, 64–72.

(363) Radway, J. C.; Weissman, J. C.; Wilde, E. W.; Benemann, J. R. Exposure of *Fischerella* [*Mastigocladus*] to high and low temperature extremes: strain evaluation for a thermal mitigation process. *J. Appl. Phycol.* 1992, *4*, 67–77.

(364) Yilmaz Ozturk, B.; Asikkutlu, B.; Akkoz, C.; Atici, T. Molecular and Morphological Characterization of Several Cyanobacteria and Chlorophyta Species Isolated from Lakes in Turkey. *Turk. J. Fish. Aquat. Sci.* 2019, *19*, 635–643.

(365) Vaishampayan, A.; Sinha, R. P.; Hader, D.-P.; Dey, T.; Gupta, A. K.; Bhan, U.; Rao, A. L. Cyanobacterial biofertilizers in rice agriculture. *Bot. Rev* 2001, *67*, 453–516.

(366) Banerjee, M. Comparative study on phosphatase activity of four rice field cyanobacterial strains and their ecological implications. *Nova Hedw.* 2007, *85*, 407–416.

(367) Sharma, L. K.; Zaeen, A. A.; Bali, S. K.; Dwyer, J. D. Improving Nitrogen and Phosphorus Efficiency for Optimal Plant Growth and Yield. In *New visions in plant science*; Çelik, Ö., Ed.; IntechOpen: London, 2018.

(368) Sandhiya, G. S.; Sugitha, T. C. K.; Balachandar, D.; Kumar, K. Endophytic colonization and in planta nitrogen fixation by a diazotrophic *Serratia* sp. in rice. *Indian J. Exp. Biol.* 2005, *43*, 802–807.

(369) Nascente, A. S.; Lanna, A. C.; Sousa, T. P. de; Chaibub, A. A.; Souza, A. C. A. de; Filippi, M. C. C. de. N Fertilizer Dose-Dependent Efficiency of *Serratia* spp. for Improving Growth and Yield of Upland Rice (*Oryza sativa* L.). *Int. J. Plant Prod.* 2019, *13*, 217–226.

(370) Someya, N.; Nakajima, M.; Watanabe, K.; Hibi, T.; Akutsu, K. Influence of bacteria isolated from rice plants and rhizospheres on antibiotic production by the antagonistic bacterium *Serratia marcescens* strain B2. *J. Gen. Plant Pathol.* 2003, *69*, 342–347.

(371) Chittora, D.; Meena, M.; Barupal, T.; Swapnil, P. Cyanobacteria as a source of biofertilizers for sustainable agriculture. *Biochem. Biophys. Rep.* 2020, *22*, 100737.

(372) Ludueña, L. M.; Anzuay, M. S.; Angelini, J. G.; McIntosh, M.; Becker, A.; Rupp, O.; Goesmann, A.; Blom, J.; Fabra, A.; Taurian, T. Strain *Serratia* sp. S119: A potential biofertilizer for peanut and maize and a model bacterium to study phosphate solubilization mechanisms. *Appl. Soil Ecol.* 2018, *126*, 107–112.

(373) Flores, E.; Wolk, C. P. Production, by filamentous, nitrogen-fixing cyanobacteria, of a bacteriocin and of other antibiotics that kill related strains. *Arch. Microbiol.* 1986, *145*, 215–219.

(374) Schlegel, I.; Doan, N. T.; Chazal, N. de; Smith, G. D. Antibiotic activity of new cyanobacterial isolates from Australia and Asia against green algae and cyanobacteria. *J. Appl. Phycol.* 1998, *10*, 471–479.

(375) Gantar, M.; Berry, J. P.; Thomas, S.; Wang, M.; Perez, R.; Rein, K. S. Allelopathic activity among Cyanobacteria and microalgae isolated from Florida freshwater habitats. *FEMS Microbiol. Ecol.* 2008, *64*, 55–64.

(376) Hagmann, L.; Jüttner, F. Fischerellin A, a Novel Photosystem-II-inhibiting Allelochemical of the Cyanobacterium *Fischerella muscicola* with Antifungal and Herbicidal Activity. *Tetrahedron Lett.* 1996, *37*, 6539–6542.

(377) Papke, U.; Gross, E. M.; Francke, W. Isolation, identification and determination of the absolute configuration of Fischerellin B. A new algicide from the freshwater cyanobacterium *Fischerella muscicola* (Thuret). *Tetrahedron Lett.* 1997, *38*, 379–382.

(378) Doan, N. T.; Stewart, P. R.; Smith, G. D. Inhibition of bacterial RNA polymerase by the cyanobacterial metabolites 12- epi -hapalindole E isonitrile and calothrixin A. *FEMS Microbiol. Lett.* 2001, *196*, 135–139.

(379) Etchegaray, A.; Rabello, E.; Dieckmann, R.; Moon, D. H.; Fiore, M. F.; Döhren, H. von; Tsai, S. M.; Neilan, B. a. Algicide production by the filamentous cyanobacterium *Fischerella* sp. CENA 19. *J. Appl. Phycol.* 2004, *16*, 237–243.

(380) Srivastava, A.; Jüttner, F.; Strasser, R. J. Action of the allelochemical, fischerellin A, on photosystem II. *Biochim. Biophys. Acta, Bioenerg.* 1998, *1364*, 326–336.

(381) Jeong, H.; Yim, J. H.; Lee, C.; Choi, S.-H.; Park, Y. K.; Yoon, S. H.; Hur, C.-G.; Kang, H.-Y.; Kim, D.; Lee, H. H.*; et al.* Genomic blueprint of *Hahella chejuensis*, a marine microbe producing an algicidal agent. *Nucleic Acids Res.* 2005, *33*, 7066–7073.

(382) Zhang, H.; Peng, Y.; Zhang, S.; Cai, G.; Li, Y.; Yang, X.; Yang, K.; Chen, Z.; Zhang, J.; Wang, H.*; et al.* Algicidal Effects of Prodigiosin on the Harmful Algae *Phaeocystis globosa*. *Front. Microbiol.* 2016, *7*, 602.

(383) Zhang, H.; Wang, H.; Zheng, W.; Yao, Z.; Peng, Y.; Zhang, S.; Hu, Z.; Tao, Z.; Zheng, T. Toxic Effects of Prodigiosin Secreted by *Hahella* sp. KA22 on Harmful Alga *Phaeocystis globosa*. *Front. Microbiol.* 2017, *8*, 999.

(384) Yang, K.; Chen, Q.; Zhang, D.; Zhang, H.; Lei, X.; Chen, Z.; Li, Y.; Hong, Y.; Ma, X.; Zheng, W.*; et al.* The algicidal mechanism of prodigiosin from *Hahella* sp. KA22 against *Microcystis aeruginosa*. *Sci. Rep.* 2017, *7*, 7750.

(385) DAVIDSON, F. F. Effects of extracts of blue-green algae on pigment production by *Serratia marcescens*. *J. Gen. Microbiol.* 1959, *20*, 605–611.

(386) Dierstein, R.; Kaiser, I.; Weckesser, J. Inhibition of Prodigiosin Formation in *Serratia marcescens* by Extracts of Toxic Cyanobacteria. *Syst. Appl. Microbiol.* 1989, *12*, 244–248.

(387) Griffith, A. W.; Gobler, C. J. Harmful algal blooms: A climate change co-stressor in marine and freshwater ecosystems. *Harmful Algae* 2020, *91*, 101590.

Curriculum vitae

Name	Tomasz Chilczuk
Date of birth	1. September 1989
Place of birth	Opole

Doctoral degree

2016 – currently	PhD student in natural product research in the RG Pharmacognosy, under the supervision of Prof. Dr. Timo Niedermeyer, at the University of Tübingen first and at the Martin-Luther-University Halle-Wittenberg afterwards

Master of Science

2013 – 2016	Master thesis in microbiology at the department of Microbiology/Biotechnology, University of Tübingen on "Screening for quorum sensing inhibitors from cyanobacteria" under the supervision of Prof. Dr. Timo Niedermeyer

Bachelor of Science

2010 – 2013	Bachelor thesis in biology at the department of Microbiology/Organismic Interactions, University of Tübingen on "Herstellung eines FRET-basierten Biosensors für die Metaboliten ADP, ATP und 2-Oxoglutarat" under the supervision of Prof. Dr. Karl Forchhammer

Civilian service

2009 – 2010	Completion of the civilian service at the Caritasverband Mannheim e.V., Mannheim

School

2007 – 2009	Acquisition of the general qualification for university entrance at the IGMH Gesamtschule, Mannheim
2000 – 2007	Moll-Gymnasium, Mannheim
1995 – 2000	Rheinauer-Grundschule, Mannheim

Date

Tomasz Chilczuk

List of publications and contributions

Publication 1:

Halogenation-Guided Chemical Screening Provides Insight into Tjipanazole Biosynthesis by the Cyanobacterium *Fischerella ambigua*

Tomasz Chilczuk, Till F. Schäberle, Sahel Vahdati, Ute Mettal, Mustafa El Omari, Heike Enke, Michael Wiese, Gabriele M. König, Timo H. J. Niedermeyer

ChemBioChem 2020, *21*, 2170–2177.

DOI: 10.1002/cbic.202000025

Author contributions:

Author	Scientific ideas [%]	Data generation [%]	Analysis & interpretation [%]	Paper writing [%]
Tomasz Chilczuk	40	45	30	50
Till F. Schäberle	15	15	10	20
Sahel Vahdati	0	15	5	0
Ute Mettal	5	10	10	0
Mustafa El Omari	5	10	10	0
Heike Enke	0	5	0	0
Michael Wiese	0	0	5	5
Gabriele M. König	10	0	10	5
Timo H. J. Niedermeyer	25	0	20	20

Publication 2:

Hapalindoles from the Cyanobacterium *Hapalosiphon* sp. Inhibit T Cell Proliferation

Tomasz Chilczuk, Carmen Steinborn, Steffen Breinlinger, Amy M. Zimmermann-Klemd, Roman Huber, Heike Enke, Dan Enke, Timo H. J. Niedermeyer, Carsten Gründemann

Planta Medica 2020, *86*, 96–103.

DOI: 10.1055/a-1045-5178

Author contributions:

Author	Scientific ideas [%]	Data generation [%]	Analysis & interpretation [%]	Paper writing [%]
Tomasz Chilczuk	10	30	15	20
Carmen Steinborn	10	20	25	20
Steffen Breinlinger	10	20	30	20
Amy M. Zimmermann-Klemd	0	10	0	0
Roman Huber	0	10	0	0
Heike Enke	0	5	0	0
Dan Enke	0	5	0	0
Timo H. J. Niedermeyer	35	0	15	20
Carsten Gründemann	35	0	15	20

Publication 3:

Ambigols from the Cyanobacterium Fischerella ambigua Increase Prodigiosin Production in Serratia spp

Tomasz Chilczuk, Rita Monson, Peter Schmieder, Vesselin Christov, Heike Enke, George Salmond, Timo H. J. Niedermeyer

ACS Chemical Biology 2020, *15*, 2529–2936.

DOI: 10.1021/acschembio.0c00554

Author contributions:

Author	Scientific ideas [%]	Data generation [%]	Analysis & interpretation [%]	Paper writing [%]
Tomasz Chilczuk	40	60	50	50
Rita Monson	20	5	10	15
Peter Schmieder	0	20	10	5
Vesselin Christov	0	10	0	0
Heike Enke	0	5	0	0
George Salmond	0	0	5	0
Timo H. J. Niedermeyer	40	0	25	30

Danksagung

An dieser Stelle bedanke ich mich, bei…

Prof. Dr. Timo Niedermeyer für die tolle Betreuung dieser Arbeit, sein Verständnis für meine vielen Fragen und die Gewährung großer wissenschaftlicher Freiräume für ein selbständiges Arbeiten.

Prof. Dr. Markus Nett und Prof. Dr. Björn Junker für die Begutachtung meiner Arbeit.

Dr. Andrea Porzel und Dr. Peter Schmieder für die Durchführung zeitaufwendiger NMR-Messungen sowie für die Unterstützung bei der Datenauswertung.

Dr. Dan Kramer und der Cyano Biotech GmbH in Berlin für die Bereitstellung der im Rahmen dieser Arbeit untersuchten Cyanobakterienextrakte.

Prof. Dr. George Salmond und Dr. Rita Manson für die Unterstützung meines Projektes und die Gastfreundlichkeit während meines Aufenthaltes in Cambridge.

dem GRK 1708 für die finanzielle Förderung eines Teils der Arbeiten.

meinem Kollegen Nico Ortlieb für das Korrekturlesen dieser Arbeit und die lustige Zeit auf Konferenzen und im Labor.

meinen Kolleginnen und Kollegen Julia, Paul, Ronja, Steffen für ihre Hilfsbereitschaft, die gute Zusammenarbeit und freundliche Arbeitsatmosphäre – wir waren ein tolles Team.

Nadine Silber und Dhana Thomy für die Hilfsbereitschaft und Einweisung ins Labor der AG Brötz-Oesterhelt. Ich habe mich immer darauf gefreut euch zu besuchen.

meiner Familie und meiner Freundin für ihr Verständnis und die unendliche Unterstützung während der letzten Jahre.

Eidesstattliche Erklärung

Ich versichere hiermit, dass ich die vorliegende Dissertation selbständig und ohne unzulässige fremde Hilfe angefertigt habe. Ich habe keine anderen als die im Literaturverzeichnis angeführten Quellen genutzt und sämtliche Textstellen, die wörtlich oder sinngemäß aus veröffentlichten oder unveröffentlichten Schriften entnommen wurden, und alle Angaben, die auf mündlichen Auskünften beruhen, als solche kenntlich gemacht. Alle von anderen Personen bereitgestellten Materialien oder erbrachten Dienstleistungen wurden als solche gekennzeichnet.

Die vorgelegte Arbeit ist weder im Inland noch im Ausland in gleicher oder ähnlicher Form einer anderen Prüfungsbehörde zum Zweck einer Promotion oder eines anderen Prüfungsverfahrens vorgelegt worden. Ich habe keine früheren erfolglosen Promotionsversuche unternommen.

Datum	Tomasz Chilczuk