ChemInform Abstract: Naturally Occurring Tetramic Acid Products: Isolation, Structure Elucidation and Biological Activity

ARTICLE *in* RSC ADVANCES · SEPTEMBER 2014

Impact Factor: 3.84 · DOI: 10.1039/C4RA09047K

3 AUTHORS, INCLUDING:

Mo Xuhua

6 PUBLICATIONS **45** CITATIONS

SEE PROFILE

Jianhua Ju

Chinese Academy of Sciences

85 PUBLICATIONS **1,557** CITATIONS

SEE PROFILE

RSC Advances

REVIEW

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2014, **4**, 50566

Naturally occurring tetramic acid products: isolation, structure elucidation and biological activity

Xuhua Mo,[ab] Qinglian Li[b] and Jianhua Ju*[b]

Received 21st August 2014
Accepted 26th September 2014

DOI: 10.1039/c4ra09047k

www.rsc.org/advances

Natural products containing the tetramic acid core scaffold have been isolated from an assortment of terrestrial and marine species and often display wide ranging and potent biological activities including antibacterial, antiviral and antitumoral activities. Owing to their intriguing structure and biological activity, tetramic acid-containing agents, both natural and synthetic, are attracting increasingly significant attention from biologists and chemists. Indeed, this increasing enthusiasm has led to significant advances. The goal of this review is to present not only these advances but also the broader context that frames them and provides a complete view of the naturally occurring tetramic acids inclusive of studies aimed at isolation, structure elucidation, and evaluation of biological activities. Consistent with advances over the past decade this review covers the period spanning 2002 to 2013.

1. Introduction

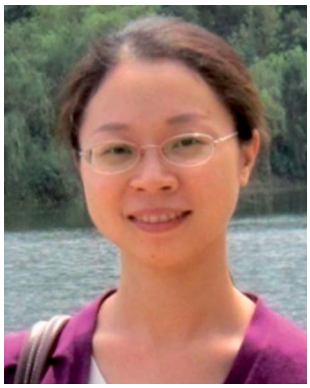
Natural products containing the tetramic acid structure (pyrrolidine-2,4-dione) are isolated from various terrestrial and marine organisms; often they are metabolites originating from bacteria, fungi and assorted sponges. These structures can be characterized as simple heterocycles or more complex systems possibly containing long chains or fused polycyclic skeletons. Their level of structural complexity can vary dramatically and consistent with this diversity compounds comprising this family display a broad range of biological activities; antimicrobial, antitumor and antiviral activities have been noted for numerous family members. Due to their intriguing structures and associated biological activities, tetramic acid-containing natural products are gaining more and more attention from both the biological and chemical communities.

As of 2003, several reviews on the subjects of tetramic acid compound isolation, biological activity, chemical synthesis and biosynthesis have been published by Royles, Ghisalberti and Gossauer.[1–3] In the last decade, numerous tetramic acid compounds have been isolated from a myriad of diverse environments and studies on tetramic acid biosynthetic pathways

[a]*Shandong Key Laboratory of Applied Mycology, School of Life Sciences, Qingdao Agricultural University, Qingdao, Shandong 266109, China*

[b]*CAS Key Laboratory of Tropical Marine Bio-resources and Ecology, Guangdong Key Laboratory of Marine Materia Medica, RNAM Center for Marine Microbiology, South China Sea Institute of Oceanology, Chinese Academy of Sciences, 164 West Xingang Rd., Guangzhou 510301, China. E-mail: jju@scsio.ac.cn*

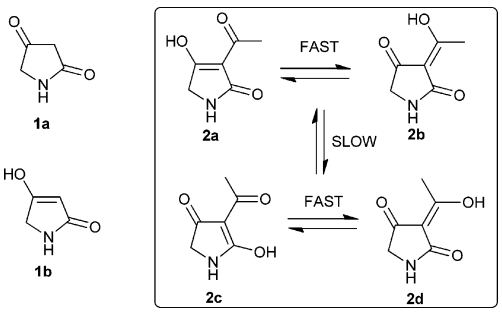
Xuhua Mo was born in Hunan Province (China) in 1984. He received his Ph.D. in 2012 from the South China Sea Institute of Oceanology, Chinese Academy of Sciences, working on marine natural products discovery and biosynthesis, under the supervision of Prof. Jianhua Ju. He is currently a lecturer involved in teaching biochemistry at Qingdao Agricultural University, China. His research interests include secondary metabolites as well as the biosynthetic pathways endogenous to many actinomycetes.



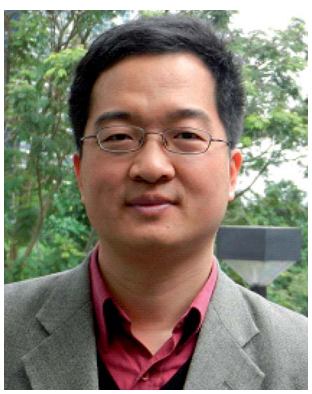
Qinglian Li was born in 1985 in Guangxi Province, China. She received her Ph.D. in microbiology and biochemical pharmacy from Peking Union Medical College in 2013. She recently joined the group of professor Jianhua Ju as an assistant researcher at the South China Sea Institute of Oceanology, Chinese Academy of Sciences, working on the biosynthetic pathway and regulation of secondary metabolites in marine Streptomycetes.

have been carried out. However, during this period, only Schobert *et al.* have provided a rigorous review on the bioactivity and biosynthesis of tetramate,[4] Hertweck *et al.* have discussed fungal PKS-NRPS biosynthesis,[5] and Fisch *et al.* has discussed microbial iterative hybrid PKS-NRPS.[6] Herein, we focus attention on the isolation, structure elucidation initiatives and efforts to decipher biological activities of more recently isolated members of the tetramic natural products family. Also discussed are specific examples of structural revisions, newly established stereochemistry, newly synthesized congeners, and new biological properties of previously reported compounds. In this review, the large group of cytochalasins now known to result from a putative tetramic acid intermediate en route to completion of their biosynthesis will be excluded. The material discussed in this review was originally reported in literature spanning the period from 2002 to 2013.

2. General aspects of the tetramic acid scaffold



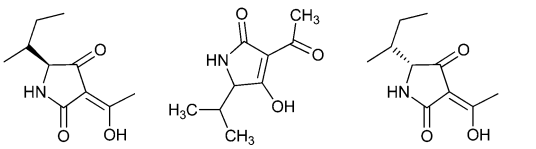
Many of the characteristics of the tetramic acid scaffold have been previously reviewed.[1–4] Consequently, we describe the scaffold only briefly here. Most naturally occurring tetramic acid compounds result from mixed polyketide synthase and non-ribosomal peptide synthetase pathways. Tetramic acid compounds are derivatives of either the keto form **1a** (pyrrolidine-2,4-dione) or the corresponding enolic form **1b** (4-hydroxy-3-pyrrolin-2-one); keto version **1a** is typically the predominant species. The 3 position in the tetramic acid ring is often substituted by an acyl moiety, and usually, the structures found at position 5 are derived from the amino acid precursors processed biosynthetically to ultimately afford the tetramic acid moiety. For 3-acyltetramic acid, four tautomers, involving two sets of rapidly interconverting internal tautomers **2a**/**2b** and **2c**/**2d**, are normally detected in solution. Arising from C–C bond rotation of the acyl side chain, the interchange of two pairs of external tautomers is slow on the NMR time scale. Interestingly, 3-acyltetramic acid can coordinate to assorted metal ions such as Fe^{3+}, Zn^{2+}, Cu^{2+}, thus generating liganded metal complexes. In some cases, metal chelation is absolutely crucial to the bioactivity of the tetramic acid compound. For example, the tetramate-based natural product harzianic acid is a type 2A serine/threonine phosphatase inhibitor, but only when present as the natural product–Zn^{2+} complex; Zn^{2+} removal abolishes all inhibitory activity.[7]



Jianhua Ju was born in 1972 in Shandong Province, China. He received his B.S. degree in pharmacy from Shandong University in 1995 and obtained his Ph.D. in natural product Chemistry at Peking Union Medical College in 2000. He then worked in the area of natural products discovery as an assistant researcher at the Chinese Academy of Medical Sciences from 2000 to 2003. He subsequently worked in professor Ben Shen's group as a research associate in the field of natural products biosynthesis from 2003 to 2008 at the University of Wisconsin–Madison, WI, USA. In 2008, he joined the South China Sea Institute of Oceanology, Chinese Academy of Sciences as a full professor working in the field of bioactive marine microbial natural products discovery and biosynthesis.

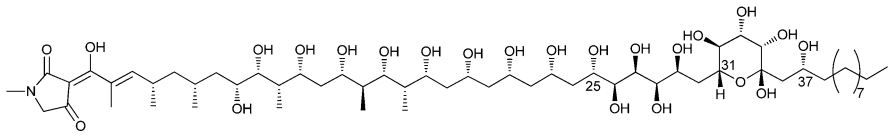
3. Advancements in tetramic acid products: isolation, structure elucidation and biological activity

3.1 Simple 3-acyl tetramic acid

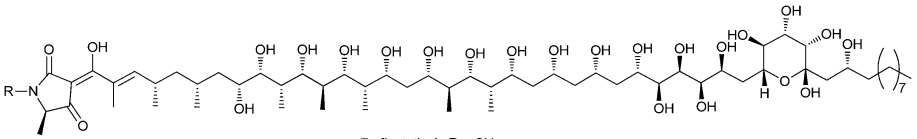


The phytotoxin L-tenuazonic acid (**3**) was first isolated from the phytopathogenic fungi *Alternaria alternate*, the causative agent behind brown leaf spot of *Eupatorium adenophorum*.[8] The related analogue 3-acetyl-5-isopropyltetramic acid (3-AIPTA, **4**) was isolated from the same strain by supplementing the culture with precursor L-valine.[9] Subsequently, **3** was also isolated from *Aspergillus* sp., *A. tenuissima*, *Pyricularia oryzae* and *Ulocladium* sp. HKI0226.[10–14] In 2004, **3** and **4** along with D-*allo*-tenuazonic acid (**5**) were re-discovered from two fungal strains *A. brassicicola* and *A. raphani* originating from pollen collected from beehives, which showed inhibitory activity against the causative agent of American foulbrood *Paenibacillus larvae*.[15] Further bioassays revealed that **5** accounts for the activity against *P. larvae* (MIC = 32 μg mL^{-1}), and displays activity comparable to the antibiotic oxytetracycline currently used to control American foulbrood.[15] The two other compounds (**3** and **4**) were only slightly active (MICs >100 μg per disk).[15] Compounds **3** and **4** were also found to inhibit root and shoot growth rates and **4** displayed phytotoxicity against a wide range of plants. Compound **4** also was found to kill seedlings of both mono- and

dicotyledonous weeds.[16–20] Both **3** and **4** block electron flow from Q_A to Q_B at photosystem II acceptor sides and studies with D1-mutants of *Chlamydomonas reinhardtii* revealed that the Gly_{256} amino acid residue plays a key role in tenuazonic acid binding to the Q_B-niche.[18,19] Further investigations revealed that **3** and **4** cause host plant cell necrosis as a result of direct oxidative damage from chloroplast-mediated reactive oxygen species.[19,20]



6 blasticidin A

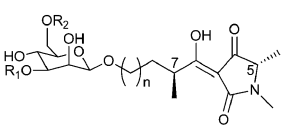


7 aflastatin A: R = CH_3
8 aflastatin B: R = H

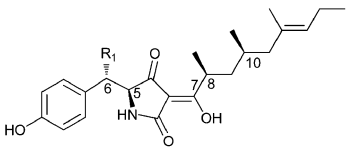
Blasticidin A (**6**), a tetramic acid derivative with a highly oxygenated long alkyl chain was obtained from the broth of *Streptomyces griseochromogenes* in 1955 although its structure and absolute configuration was not clearly characterized until nearly 50 years later.[21–24] Aflastatins A and B (**7** & **8**), compounds similar to **6**, were isolated from the broth of *Streptomyces* sp. MRI142 in 1997; the absolute configuration of **7** was completely determined in 2007.[22–25] Notably, **6** and **7** are potent inhibitors of aflatoxin production by *Aspergillus parasiticus*. Blasticidin A has been shown to significantly reduce expression of genes encoding aflatoxin biosynthesis and a key regulatory protein, AflR, leading to dramatic reductions in the abundance of aflatoxin biosynthetic enzymes in the microbial producer.[22,26,27] At low concentrations, blasticidin A inhibits aflatoxin production in *A. parasiticus* without significantly affecting fungal growth. However, at high concentrations, blasticidin A does display antifungal activity.[26] In addition, blasticidin A displays activity against *Saccharomyces cerevisiae* by inhibiting protein synthesis in a galactose-induced expression system.[26,27]

Epicoccamide (**9**) has thus far failed to show antimicrobial or cytotoxity activity although epicoccamides B–D have demonstrated antiproliferative activity against cell lines L-929, K-562 and HeLa cells.[28,29] Epicoccamide D is the most active against HeLa (CC_{50} = 17.0 μM), L-929 (GI_{50} = 50.5 μM) and K-562 (GI_{50} = 33.3 μM). Furthermore, epicoccamide D induces morphogenesis and pigment formation in surface cultures of the fungus *Phoma destructia* at a concentration of 1.7 mM.[29]

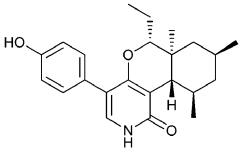
Epicoccarines A and B (**13**, **14**), along with epipyridone (**15**), were isolated from filamentous endofungal *Epicoccum* sp. associated with the tree fungus *P. squarrosa*.[31] The structures of **13** and **14** were elucidated on the basis of multiple spectroscopic data. The orientation of H-5 and H-6 in **14** was determined as *trans* on the basis of the observed $J_{H\text{-}5,H\text{-}6}$ coupling constant. The relative configuration of C-8 and C-10 was assigned to be the same as in epipyridone A (**15**), on the basis of a putative mutual biosynthetic pathway. The C-8 and C-10 assignments, as well as the 5*S* configuration were ultimately confirmed by total synthesis.[32] Epicoccarine B displayed moderate activity against Gram-positive bacteria.[31] However, epicoccarine A showed



9 epicoccamide: R_1=H, R_2=H, n=13
10 epicoccamide B: R_1=Ac, R_2=H, n=13
11 epicoccamide C: R_1=H, R_2=Ac, n=13
12 epicoccamide D: R_1=H, R_2=H, n=15



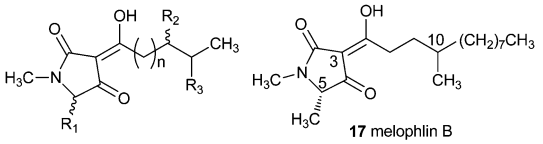
13 epicoccarine A: R_1 = H
14 epicoccarine B: R_1 = OH



15 epipyridone

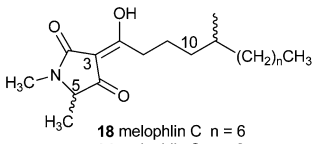
Epicoccamide (**9**) was first isolated from cultures of the fungus *Epicoccum purpurascens* originating from the inner tissue of the jellyfish *Aurelia aurita* collected in the North Sea in 2001.[28] The compound was re-isolated as the major metabolite along with epicoccamides B–D (**10**–**12**) from the endofungal *Epicoccum* sp. associated with the tree fungus *Pholiota squarrosa* in 2008.[29] Epicoccamides consist of three biosynthetically distinct subunits: a β-D-mannose, an aliphatic chain, and a tetramic acid moiety, which differ in their mannose substitution patterns and the length of their aliphatic chains. Epicoccamide was found to be a 2 : 3 mixture of 4(*R*) and 4(*S*)-isomers; the 5*S*, 7*S* stereochemistry of the epicoccamides were ultimately elucidated by the application of total synthesis.[30]

selective activity against *Mycobacterium vaccae* (MIC = 6.25 μg mL^{-1}) and weak activity against multidrug resistant *Streptococcus aureus* 134/94 (MIC = 50 μg mL^{-1}) and *Mycobacterium fortuitum* B (MIC = 50 μg mL^{-1}).[31]



17 melophlin B

16 melophlin A R_1 = H, R_2 = H, R_3 = H, n = 12
19 melophlin D R_1 = H, R_2 = H, R_3 = H, n = 11
20 melophlin E R_1 = H, R_2 = H, R_3 = CH_3, n = 11
21 melophlin F R_1 = H, R_2 = CH_3, R_3 = H, n = 11
22 melophlin G R_1 = H, R_2 = H, R_3 = H, n = 10
23 melophlin H R_1 = H, R_2 = H, R_3 =CH_3, n = 10
24 melophlin I R_1 = H, R_2 = CH_3, R_3 = H, n = 10
25 melophlin J R_1 = CH_3, R_2 = H, R_3 = H, n = 10
26 melophlin K R_1 = H, R_2 = H, R_3 = CH_3, n = 9
27 melophlin L R_1 = CH_3, R_2 = H, R_3 = H, n = 9
28 melophlin M R_1 = CH_3, R_2 = H, R_3 = H, n = 8
29 melophlin N R_1 = CH_3, R_2 = H, R_3 = CH_3, n = 8
30 melophlin O R_1 = CH_3, R_2 = CH_3, R_3 = H, n = 8
31 melophlin P R_1 = CH_3, R_2 = H, R_3 = H, n = 12
32 melophlin Q R_1 = CH_3, R_2 = H, R_3 = CH_3, n = 10
33 melophlin R R_1 = H, R_2 =CH_3, R_3 = H, n = 10

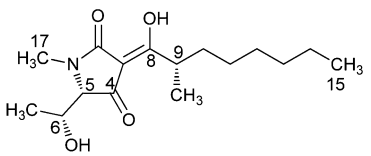


18 melophlin C n = 6
34 melophlin S n = 8

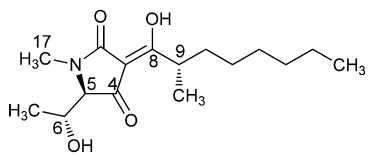
Melophlins, as characterized by their *N*-methyl-3-acyltetramic acid scaffold, were originally isolated from marine sponges of the genus *Melophlus*, and differ in what substituent resides at the C-5 and C-3 centers of the pyrrodine-2,4-dione core. Melophlins A and B (**16**, **17**) and melophlins C–O (**18–30**), were discovered from the marine sponge *Melophlus sarassinorum* collected from various regions in Indonesia.[33,34] Subsequently, melophlins A, D, E, G, H, I, together with four new compounds, melophlins P–S (**31–34**), were identified from two marine sponges of the *Melophlus* genus collected in Palau.[35] Melophlin A–C and P–S were found to exist as a 9 : 1 mixtures of exo A- and exo B forms.[33–35] Except for melophlin B, which has a 5*S* configuration, melophlins C, J, L–Q and S have all been found to exist as mixtures of the 5(*S*) and 5(*R*)-enantiomers.[33–35] In 2008, Biersack *et al.* accomplished the total synthesis of melophlins P, Q and R in four steps and in so doing, confirmed a number of earlier stereochemical hypotheses.[36]

This class of compounds has demonstrated cytotoxicity toward a series of cell lines (Table 1).[33–38] In addition, melophlins A and B have been found to display cytotoxic activity against HL60, HeLa and TF-1 cells and also are capable of phenotypically reverting *ras*-transformed NIH3T3 cells to the non-transformed "normal" state at concentrations of 5 μg mL^{-1}.[33] Moreover, **16** and **17** both arrest NIH3T3 cells in the G1 phase at 1 μg ML^{-1}.[33] Furthermore, antibacterial activity have been observed from melophlin B, C, P, Q and R.[34–36,39] Very significantly, the biological activity of the melophlins can be modulated by metal coordination events (Table 1).[39] Importantly, improvements in both cytotoxicity and antibacterial activities are apparent upon metal complexation events with assorted melophlins.[39]

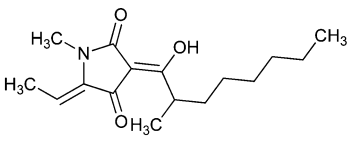
Six penicillenols A_1, A_2, B_1, B_2, C_1, and C_2 (**35–40**) were discovered from the endophytic fungus *Penicillium* sp. GQ-7 associated with *Aegiceras corniculatum* by Lin *et al.* in 2008.[40] The *5S* configuration in penicillenols A_1 and C_1 and *5R* configuration in penicillenols A_2 and C_2 were assigned on the basis of comparisons of CD spectra with tenuazonic acid, and the 5*S*, 6*R*, 9*S* absolute configuration of penicillenol A_1 and the 5*R*, 6*R*, 9*S* absolute configurations of penicillenol A_2 and penicillenol C_1 were confirmed by total synthesis.[40–43] An evaluation of cytotoxicities against A-549, BEL-7402, P388 and HL-60 cell lines revealed that penicillenol A_1 exerts the most potent activity against the four cell lines; IC_{50} values of 23.8 μM, 13.03 μM,



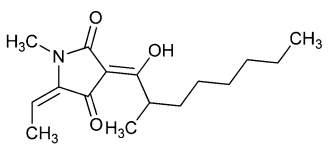
35 penicillenol A_1



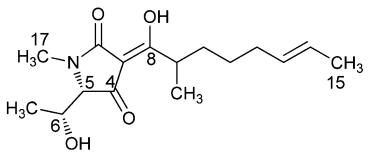
36 penicillenol A_2



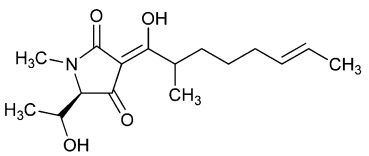
37 penicillenol B_1



38 penicillenol B_2



39 Penicillenol C_1

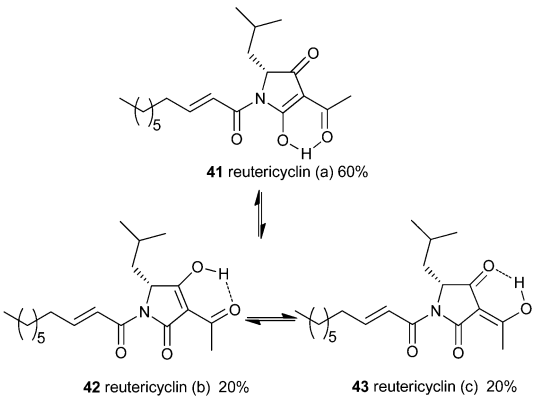


40 penicillenol C_2

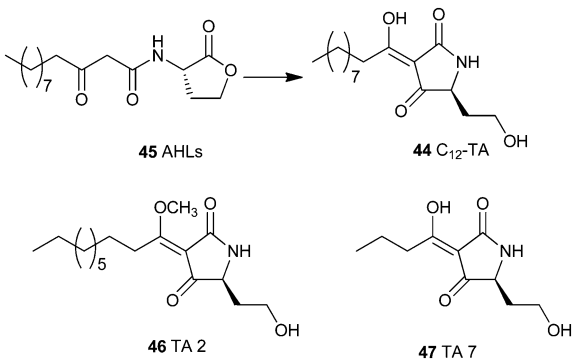
Table 1 Summary of cytotoxicities for the melophlins against assorted cancer cell lines (μM)

Melophlin	V79 (EC_{50})	L1210 (IC_{50})	KB-3-1 (IC_{50})	A-498 (IC_{50})	U-937 (IC_{50})	L929 (IC_{50})
A	27.2	8.55	26 ± 0.4	21 ± 1.6	8.8 ± 0.4	24 ± 2.0
B			9.3 ± 0.4	6.8 ± 0.4	3.3 ± 0.1	11 ± 2.0
C			3.6 ± 0.2	3.7 ± 0.0	2.6 ± 0.9	3.4 ± 0.1
D	>50	38.6				
E	19.8	28.5				
G	>50	>50	22 ± 0.2	14 ± 2.2	6.7 ± 0.2	22 ± 1.1
H	8.5	16.3				
I	23.1	7.12				
O	9.6	1.86				
P	>50	20.0	17 ± 2.1	15 ± 1.8	2.1 ± 0.2	13 ± 0.4
Q	44.0	10.5	16 ± 1.8	3.1 ± 0.4	3.6 ± 1.1	19 ± 0.1
R	13.3	0.85	5.9 ± 0.2	2.1 ± 1.0	2.2 ± 0.6	6.1 ± 0.1
S	16.7	5.13				
Ca-$(A)_2$			10 ± 1.0	9.0 ± 0.6	6.1 ± 0.9	8.3 ± 0.4
Mg-$(A)_2$			12	8.3	5.4	12
Zn-$(A)_2$			10	9.8	3.9	12
Ga-$(C)_3$			7.7	2.7	4.0	7.7
La-$(C)_3$			1.9	0.54	0.4	2.5

8.85 μM and 0.76 μM, respectively were noted for penicillenol A_1.[40] Penicillenols A_2, B_1, and B_2 displayed cytotoxicity against HL-60 cell lines with IC_{50}s ranging from 3.20–16.26 μM. The difference in cytotoxicities between penicillenols A_1 and A_2 implies that the 5*S*-position is likely essential to cell killing. Although penicillenols C_1 and C_2 resemble the other four compounds, these two compounds failed to exert any noteworthy activity, implying that the saturated fatty chain at C-8 might be essential to the penicillenol pharmacophore.[40]



Reutericyclin (**41**), another tetramic acid was found to possess *N*-1 substitution with an α, β unsaturated fatty acid, and was the first low molecular weight antibiotic isolated from the broth of lactic acid bacteria *Lactobacillus reuteri* LTH2584 originating from an industrial sourdough SER.[44,45] The structure of reutericyclin was confirmed by chemical synthesis.[46,47] As indicated by NMR signals, reutericyclin exists as a mixture of three tautomers a, b and c (**41–43**) in acetonitrile solution with a stoichiometry of 60%, 20% and 20%, which is different from the other naturally occurring 3-acyltetramic acid.[44] Reutericyclin has shown potent activity against a broad range of Gram-positive bacteria and drug-resistant strains with the values of MICs in the range 0.08 to 6.25 mg mL^{-1}.[44,48–51] However, thus far, reutericyclin has failed to inhibit the growth of yeast, fungi and Gram-negative bacteria.[49] Differences in the activity against *L. sanfranciscensis* between natural reutericyclin (MIC = 0.10 mg mL^{-1}) and synthetic racemic reutericyclin (MIC = 0.75 mg mL^{-1}) indicated that stereochemistry is vital to the compounds bioactivity.[46] Notably, the outer membrane is essential for the resistance of Gram-negative to reutericyclin.[45] Although the target of reutericyclin is the cytoplasmic membrane acting as a proton ionophore to selectively dissipate transmembrane ΔpH in sensitive cells, reutericyclin has also been found to affect biofilms of *S. aureus* and *S. epidermidis*, making it a useful candidate for controlling recalcitrant biofilm-mediated skin infections.[48]



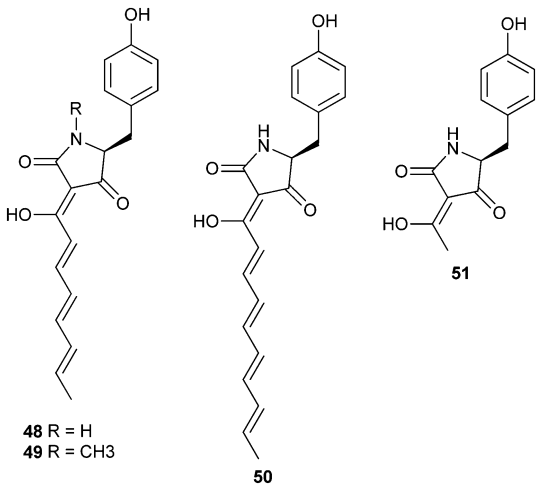
The tetramic acid compound (*S*)-3-(1-hydroxydecylidene)-5-(2-hydroxyethyl) pyrrolidine-2,4-dione (C_{12}-TA, **44**), a compound similar to **41**, was discovered as a degradation product of the quorum sensing molecule *N*-acylhomoserine lactone (AHL, **45**); it was also detected as an innate compound in cultures of *Pseudomonas aeruginosa*.[52] The mechanism for formation of **44** is a non-enzymatic Claisen-like intramolecular alkylation of the ketoamide moiety. This phenomenon of intramolecular

rearrangement is general to a variety of 3-oxo-AHLs with various acyl chain lengths.[52] The activity of **44** has been found to be more potent than that of parent AHLs, and **44** exhibits bactericidal activity against a series of Gram-positive bacteria.[52–54] Particularly noteworthy is its activity against methicillin-resistant *S. aureus* USA-300 (MIC = 25 μg mL^{-1}) and human pathogenic *M. tuberculosis* H37Rv (MIC = 5 μg mL^{-1}) as well as its apparent ability to elude or failure to trigger resistance in *S. aureus*.[52–55] Remarkably, culturing this human pathogen in the presence of subinhibitory concentrations of **44** for up to 20 passages failed to ever afford any colonies resistant to **44**.[52–55]

Compound C_{14}-TA with an acyl chain longer than in **44** showed more potent antibacterial activity than **44**, whereas synthetic compounds TA2 (**46**) and TA7 (**47**) showed less activity than **44**, suggesting the length of the acyl side chain and the keto-enol structure are both essential to activity.[53–55] In a fashion similar to that of **41**, the target of **44** is the bacterial outer membrane.[55] It appears that **44** acts as a proton ionophore which dissipates the transmembrane change in pH thereby triggering cell lysis.[55] This mechanism of cell killing closely parallels that of **41**.[48]

Like other naturally occurring tetramic acid compounds, **44** can form a C_{12}-TA-Fe^{3+} or C_{12}-TA-Ga^{3+} complex with a 3 : 1 stoichiometry; the relative affinity (K_d, app) of **44** for metal ($K_d = 1.6 \times 10^{-29}$ M^3) is stronger than that of EDTA ($K_d = 5.00 \times 10^{-23}$ M) and the siderophore pyochelin (10^{-5} M).[52] Although **44** clearly has appreciable affinity for Fe^{3+}, the iron absorbed by virtue of the **44–Fe^{3+}** complex in isolation, is not sufficient for *P. aeruginosa* growth.[56] Meanwhile, C_{12}-TA-Fe^{3+} and C_{12}-TA-Ga^{3+} complexes show the same toxic activities as the free tetramic acid, thus raising questions pertaining to the importance of **44**-metal binding in antibacterial action.[56] Importantly, **44** forms the basis for a proposed interference strategy employed by *P. aeruginosa* to preclude encroachment by competing bacteria encountered in the environment. The merits of broad-spectrum antibacterial activity, mode of action, tolerance by human cells and lack of rapid resistance might lead the scaffold represented by **44** to become a new lead for the development of new antibacterial therapeutics.

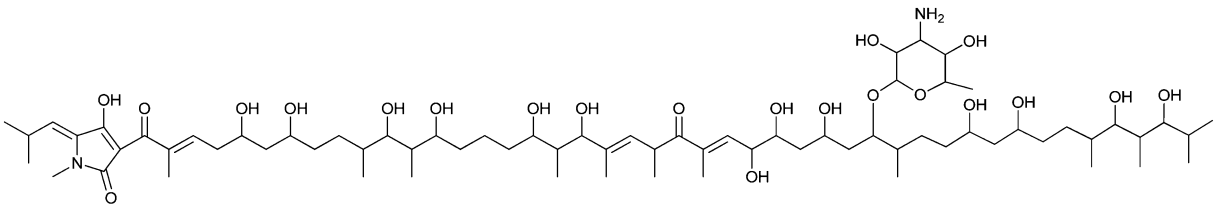
Four 3-acyltetramic acid compounds **48–51** responsible for the orange-yellow colour of myxomycetes slime mould *Leocarpus fragilis* were isolated in 1989.[57] Compounds **48**, **50** and **51**



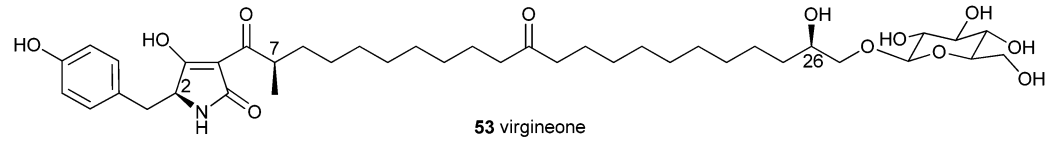
were synthesized along with a series of analogs in 2010.[58] However, no antimicrobial activity was observed when these agents were assayed against *Fusarium oxysporum*, *A. niger*, *S. aureus* and *Candida albicans*.[58]

Vancoresmycin (**52**), isolated from the broth of *Amycolatopsis* sp. ST 101170, is a tetramic acid derivative with a highly oxygenated long alkyl chain, in which an aminoglycoside moiety is linked to C-31.[59] The chemical structure of vancoresmycin was determined on the basis of a series semi-synthetic acetylation experiments and ensuing spectroscopic analyses. However, the absolute configuration has yet to be determined.[59] Notably, **52** displays more potent antibiotic activity than does vancomycin against the Gram-positive bacterium *S. aureus* including drug-resistant strains (MIC < 0.04 μg mL^{-1}), and *E. faecalis* including multidrug-resistant strains (MIC < 0.6 μg mL^{-1}).[59] Vancoresmycin is inactive against Gram-negative bacteria and assorted fungi.[59]

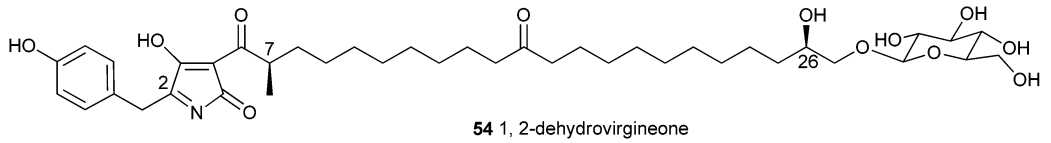
Virgineone (**53**), a tyrosine-derived tetramic acid with a C-22 oxygenated chain and a β-mannose, was first discovered from the broth of a fungus *Lachnum* V*irgineum* originating from an unidentified plant by scientists at Merck Corporation using the *Candida albicans* fitness test method to screen for biologically active species.[60] Subsequently, **53** was found to exist in a series of *Lachnum* sp. microbes isolated from a number of different



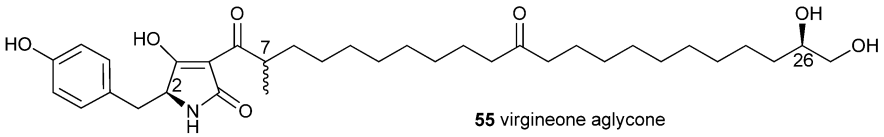
52 vancoresmycin



53 virgineone



54 1, 2-dehydrovirgineone



55 virgineone aglycone

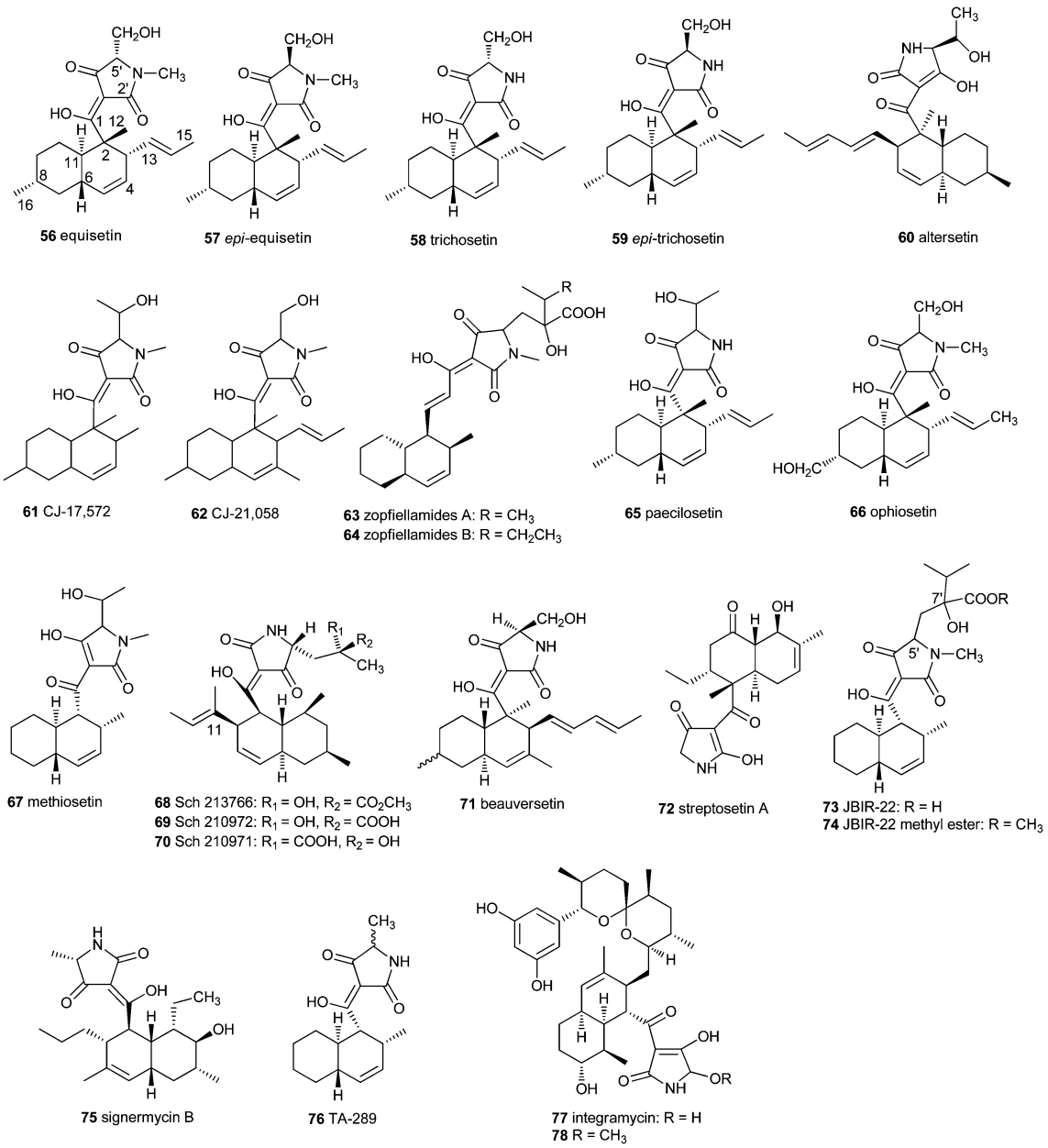
geographic locations.[60] More recently, **53** was re-isolated from the filamentous fungus *Bionectria* sp. MSX 47401 along with 1,2-dehydrovirgineone (**54**) and virgineone aglycone (**55**).[61] The regiochemical layout of the structure and the 2*S*, 7*S*, 26*S* configurations of **53** were originally determined using an assortment of spectroscopic methods and have received significantly more rigorous analysis enabled by total synthesis of the virgineone aglycone.[60,62] Interestingly, the virgineone aglycone **55** has been recently concluded to be 2*S*, 7*RS*, 26*S* on the basis of NMR data and optical rotations of synthetic and isolated **55**. It has been noted however, that generation of **55** from **53** may well lead to epimerization at C-7 thus accounting for the scrambled stereochemistry at C-7; comparisons of NMR data for synthetic materials *versus* natural **53** support the originally assigned 2*S*, 7*S*, 26*S* assignment.[62]

In vitro antimicrobial experiments have demonstrated that aglycone **55** is more potent against *S. aureus* and several related MRSA isolates (MIC = 2.8–5.5 μg mL^{-1}) than is **53** (MIC = 7.2 μg mL^{-1}) and 1,2-dehydrovirgineone (MIC = 14.0–28.0 μg mL^{-1}).[61] In addition, **53** was found to exert broad antifungal activity against *Candida* spp., *Aspergillus* spp. and *Trichophyton mentagrophytes*, displaying MICs in the range of 4–16 μg mL^{-1}.[60,61] However, the results of *in vivo* assays with a disseminated *C. albicans* MY1055 mouse model (DBA/2) revealed that **53** lacks any significant or efficacious antifungal activity.[60]

3.2 3-Decalinoyltetramic acids

Equisetin (**56**), the first member of the so-called 3-decalinoyltetramic acid family, was isolated from *Fusarium equisetin* and *F. heterosporum* and found to display extensive biological activities including antibiotic activity, HIV inhibitory activity, cytotoxicity and mammalian DNA binding capabilities.[1] In 1999 equisetin, along with *epi*-equisetin (**57**), was isolated from *F. equiseti* and *F. pallidoroseum* collected from the embryos of weathered cottonseed.[63] Subsequently in 2010, **56** was re-isolated from the mycopathogenic fungus *Elaphocordyceps ophioglossoides*.[64] It was found that **56** possesses potent antibacterial activity and very weak SecA inhibitory activity.[64,65] Furthermore, **56** and **57** have both been found to display phytotoxicity; both compounds suppressed germination or inhibited the growth of various monocotyledonous and dicotyledonous seeds as well as young seedlings and caused necrotic lesions on the roots, cotyledons, and coleoptiles of the plant seedlings evaluated.[63]

Trichosetin (**58**), the *N*-desmethyl homolog of **56**, was produced on hormone-free Murashige and Skoog medium by dual culturing of *Trichoderma harzianum* H14 and *Catharanthus roseus* callus.[66] Notably, individual cultures of *T. harzianum* H14 or *C. roseus* callus failed to produce **58** suggesting a certain level of synergy between the two organisms in terms of generating **58**.[66] Due to the similarity of the structures between **56** and **58**, **58** is hypothesized to be a metabolite produced by *T. harzianum* H14. During the course of screening for undecaprenyl pyrophosphate synthase inhibitors in 2013, **58**, together with *epi*-trichosetin (**59**), was isolated from the culture broth of *F. oxysporum* FKI-4553 by Inokoshi and co-workers.[67] Both **58** and **59** showed broad antimicrobial activities against assorted Gram-positive and Gram-negative bacteria, mycoplasma and molds.[66,67] The activities of **58** and **59** were found to be far greater against Gram-positive bacteria than against Gram-negative bacteria.[66,67] Both **58** and **59** were found to inhibit undecaprenyl pyrophosphate synthase activity in *Staphylococcus aureus* with IC_{50} values of 83 and 30 μM, respectively.[67] Moreover, **58** also showed phytotoxicity and inhibited root growth as well as the growth of all five plant species tested (*Oryza sativa*, *Vigna radiate*, *Medicago sativa*, *Capsicum frutescens* and *Lycopersicon esculentum*); damage to seedlings was permanent and the results of FDA-PI staining indicated that cells of the trichosetin-treated roots were, for the most part, dead.[16] The targets of **58** appear to be the cell membrane and mitochondria. This was confirmed by observance of a dose-dependent increase in electrolyte leakage and lipid peroxidation as well as by the



application of specific vital staining for mitochondria.[16] In addition, the results of the Ames test, *rec* assay and micronucleus test revealed that **58** is not mutagenic.[68,69]

Altersetin (**60**) was isolated from two endophytic *Alternaria* spp. strains obtained from a leaf of *Vinca minor* and a fruit of *Eonymus europaeus*; its structure and absolute configuration were determined on the basis of NMR spectroscopy and CD data.[14] *In vitro* antimicrobial assays revealed that **60** is highly active against a series of human pathogenic bacteria including *S. aureus*, *Strephylococcus pyogenes*, *S. pneumonia* with MICs in the range of 0.12–2 μg mL^{-1}.[14] Additionally, it was found that **60** exerts moderate *in vivo* activities at concentrations of 10 or 25 mg kg^{-1}; concentrations exceeding 25 mg kg^{-1} led to adverse toxic effects.[14]

CJ-17572 (**61**) was isolated as a white amorphous powder from the broth of fungal *Pezicula* sp. CL11877.[70] This natural product displayed antibacterial activities against multi-drug resistant *S. aureus* 01A1105 (MIC = 10 μg mL^{-1}), and *E. faecalis* 03A1069 (MIC = 20 μg mL^{-1}), cytotoxic activity toward HeLa cells (IC_{90} = 7.1 μg mL^{-1}) and weak inhibition of SecA (45% inhibition at 20 μg mL^{-1}).[70]

CJ-21058 (**62**) was obtained as a white powder from the broth of an unidentified fungus CL47745.[65] Notably, **62** more potently inhibited SecA activity than did either **61** or equisetin,

displaying an IC_{50} of 15 μg mL^{-1}.[65] Compound **62** also displayed antibacterial activity against *S. aureus* 01A1105 (MIC = 5 μg mL^{-1}), *E. faecalis* 03A1069 (MIC = 5 μg mL^{-1}) and cytotoxicity against HeLa cells (IC_{90} = 32 μg mL^{-1}).[65]

Zopfiellamides A (**63**) and B (**64**) were identified from fermentations of the facultative marine ascomycete *Zopfiella latipes* CBS 611.97 isolated from a soil sample from the Indian Ocean near New Delhi.[71] Compound **63** has been found to exert moderate antibacterial activity with MIC values ranging from 2–10 μg mL^{-1}, which is approximately five times greater than that of **64**, implying that the extra methyl group influences antibacterial properties.[71] Both **63** and **64** have been shown to exert antifungal activity against yeasts *Nematospora coryli* (MIC = 2 μg mL^{-1}) and *S. cerevisiae* (MIC = 2 μg mL^{-1}), yet neither compound displays cytotoxic activity against HL60, HeLa S3, L1210 and Colo-320 cell lines even at concentrations up to 100 μg mL^{-1}.[71]

Paecilosetin (**65**) was obtained from EtOAc extracts of the *Paecilomyces farinosus* fungus isolated from an infected insect larva.[72] NOESY correlation data and optical rotation analyses revealed the relative and absolute configurations of the bicyclic scaffold to be the same as in **56** and **58**. Negative circular dichroism data (CD) at 280 nm suggested an *S*-configuration at C-5′, whereas, the configuration at C-6′ remains unclear.[72] Compound **65** was found to display cytotoxic activity against murine leukemia P388 cells (IC_{50} = 3.2 μg mL^{-1}), potent antibacterial activity as well as moderate antifungal activity.[72]

Ophiosetin (**66**), together with **56**, was isolated from the broth of the mycopathogenic fungus *Elaphocordyceps ophioglossoides* isolated from soil collected at the Tsuchiyu Hot Spring in Fukushima.[64] The relative configuration of **66** was determined to be the same as that found in equisetin on the basis of NOESY correlation data. Correspondingly, the absolute configuration was tentatively assigned to be the same as in equisetin since both compounds afforded identical optical rotations.[64] Except for the weak activity against *E. faecalis* ATCC 29212 (MIC = 128 μg mL^{-1}), no antimicrobial activities were observed for **66** at the test concentrations (256 μg mL^{-1} for antibacterial, 32 μg mL^{-1} for antifungal).[64] The antimicrobial activities determined for **56**, **66** and paecilosetin suggest that modification at C-16 of the decalin moiety in ophiosetin results in a drastic decrease in biological activity.[64]

Using an antisense *Staphylococcus aureus* Fitness Test (SaFT) screening strategy enabled the unveiling of a distinct SaFT profile produced by the extract of the tropical sooty mold fungus *Capnodium* sp. (F-190679) isolated from palm leaf litter.[73] Subsequent and more detailed investigations led to the discovery of methiosetin (**67**) through application of bioassay-guided fractionation.[73] Compound **67** was found to exert only weak activity against *S. aureus* EP167 (MIC = 256 μg mL^{-1}), *Haemophilus influenzae* (MIC = 32 μg mL^{-1}), and no activity against *S. aureus* Smith, *E. faecalis*, *S. pneumoniae*, *E. coli* at 64 μg mL^{-1}.[73]

In the course of searching for novel chemokine receptor CCR-5 inhibitors, Yang *et al.* discovered Sch 210971, Sch 210972 and Sch 213766 (**68–70**) from the fungal fermentation broth of *Chaetomium globosum* collected from sterilized leaves of evergreen plants in Tucson, Arizona, USA.[74,75] Subsequently, Neumann and co-workers obtained **69** from EtOAc extracts of the endophytic ascomycete *Microdiplodia* sp. isolated from the green seaweed *Enteromorpha* sp. collected near Fehmarn Island, Baltic Sea.[76] The chemical structure and relative configuration of **69** were determined by extensive spectral and X-ray crystallographic analyses.[74] Notably, **69**, **68** and **70** are all chemokine receptor CCR-5 inhibitors with IC_{50} values of 1.2 μM, 79 nM and 8.6 μM, respectively, suggesting that the configuration and free carboxylic acid group at C-23 of the basic scaffold both play a role in receptor binding.[74,75] When tested in the CCR-2 binding assay, no inhibitory activity was observed for any of the three compounds. Additionally, **69** displayed good inhibitory activity against HLE (IC_{50} = 1.04 μg mL^{-1}), a member of the serine protease family, and antibiotic activity against *B. megaterium* (zone of inhibition ≈ 1.5 mm at a concentration of 50 μg per disk).[76] However, **69** failed to inhibit porcine cholesterolesterase, *Electrophorus electricus* acetylcholinesterase, bovine trypsin and human leukocyte elastase (IC_{50} > 30 μg mL^{-1}).[76]

Using a bioassay-guided method, Neumann *et al.* identified beauversetin (**71**), together with 2-furoic acid, 5-hydroxymethylfuran-2-carboxylic acid and depsipeptide beauvericin, from the EtOAc extracts of the marine-derived fungus *Beauveria bassiana* originating from the sponge *Myxilla incrustans* collected from the North Sea.[76] The relative configuration of **71** was determined primarily using ROESY correlations. Although the stereochemical configuration at C-8 is unclear due to the overlapping signals, it is postulated to be as in other equisetin-like compounds. The *R*-configuration at C-5′ was determined on the basis of comparisons of the CD spectra and specific optical rotations of **71** with those of phomasetin and equisetin, as well as careful interpretation of NMR experiments evaluating epimerization of **71** in pyridine.[76] Compound **71** was found to exhibit moderate cytotoxicity against a panel of six cell lines resulting in a mean IC_{50} of 3.09 μg mL^{-1} using a monopolar assay.[76] No antimicrobial activities could be identified for **71** using a panel of *B. megaterium*, *E. coli*, *Microbotryum ciolaceum*, *Euotium rubrum*, *Mycotypha microspora*, and *Chlorella fusca*.[76]

Using a histone deacetylase (HDAC) yeast assay employing the URA3 reporter gene, Amagata *et al.* discovered streptosetin A (**72**) from the broth of *Streptomyces* sp. CP13-10 during a screening of extracts of marine-derived actinomycetes to identify human class III HDAC (SIRT) inhibitors.[77] The chemical structure of **72** was determined on the basis of multiple spectroscopic analyses and X-ray diffraction data.[77] Moreover, the 4*R*, 5*R*, 9*R*, 10*S*, 13*R* configuration was determined from rigorous analyses of X-ray diffraction data.[77] This finding was confirmed using *ab initio* electronic circular dichroism spectral calculations. Compound **72** was found to exert weak inhibitory activity against yeast Sir2p (MIC = 2.5 mM), human SIRT1 (IC_{50} = 3.7 mM) and human SIRT2 (IC_{50} = 4.5 mM).[77]

JBIR-22 (**73**) was isolated from the extracts of *Verticillium* sp. f21794 fermentation broths by activity-guided fractionation.[78] Its structure, along with its 5′*S*, 7′*S* configuration was determined by analyzing multiple spectroscopic data sets as well as those generated using its methylated derivative **74**.[78] JBIR-22 is a potent inhibitor of proteasome assembling chaperone 3 (PAC3)

homodimerization (IC_{50} = 0.2 μM).[78] Compound **73** also exerts long-term cytotoxicity against the human cervical carcinoma cell line HeLa as reflected by an IC_{50} of 68 μM after 120 h.[78] Further docking studies of **73** with the PAC3 homodimer indicated that JBIR-22 can bind the active site of a key protein–protein interaction required for PAC3 homodimerization.[78]

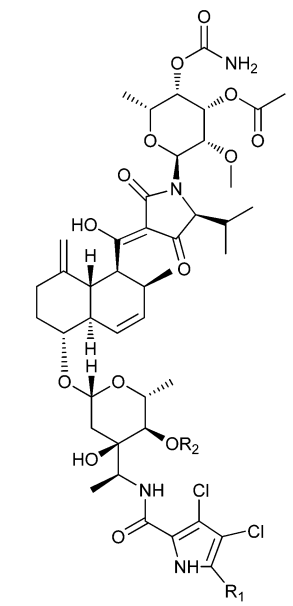
Signermycin B (**75**) was isolated from the extract of *Streptomyces* sp. strain MK851-mF8 and its structure and absolute configuration was determined on the basis of a series of spectroscopic data, X-ray crystallography, and the advanced Marfey method.[79] Natural product **75** is the first reported inhibitor of the WalK (histidine kinase)/WalR (response regulator) two-component signal (TCS) transduction system; it is potently active against Gram-positive bacteria possessing the WalK/WalR TCS including *S. aureus*, *E. faecalis* and its drug-resistant strains with MICs values ranging from 3.13–6.25 μg mL^{-1}.[79] However, **75** is ineffective against Gram-negative bacteria lacking the WalK/WalR TCS.[79] Further investigations demonstrated that **75** binds to the dimerization domain, not the ATP binding domain, of WalK with IC_{50}s in the range of 37–62 μM, and it did not compete with ATP to inhibit the autophosphorylation of WalK. The mode of action for **75** therefore involves interference with the cross-linking of WalK dimers rather than induction of protein aggregation in the presence of cross-linker.[79] This mechanism of action differs from previously reported histidine kinase inhibitors and highlights perhaps one of the more novel advances in the area of tetramic acid bioactivity studies executed over the last decade.

Tetramic acid-289 (TA-289, **76**) was isolated from the extract of unidentified fungus *Fusarium* sp.[80] and found to display inhibitory effects against *S. cerevisiae* in a pH-dependent manner. In acidic conditions (pH 3 or 4) **76** inhibits growth with MICs ranging from 10–50 μM, whereas no inhibitory activity is noted when pH = 7–8.[80] Additionally, cell death induced by **76** appears to rely on the respiratory state of the yeast cell. Moreover, TA-289 was found to cause an irreversible cell cycle block *via* microtubule-independent mechanisms.[80] Further investigations revealed that reactive oxygen species (ROS) production by the mitochondrion, combined with another unknown mechanism, leads to abnormal mitochondrial morphology ultimately limiting cell growth.[80]

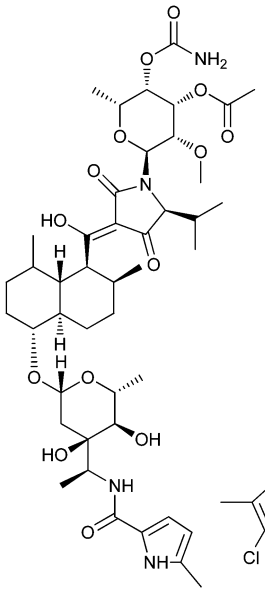
Integramycin (**77**) was discovered from the extract of *Actinoplanes* sp. (ATCC202188) by Singh *et al.*, and its structure and stereochemical configuration were elucidated following analysis of multiple spectroscopic data sets, ChemDraw three-dimensional modeling, and analysis of Dreiding models.[81] It was also determined that **77** readily reacts with methanol to produce methyl ether **78**.[81] Both **77** and **78** were found to inhibit HIV-1 integrase coupled and strand transfer reactions, although **77** is slighter more potent than is **78**; the IC_{50} values for **77** are 3 μM (for coupled transfer) and 4 μM (for strand transfer).[81]

Phillips *et al.* applied a *S. aureus* fitness test to screen crude extracts for biological activity and discovered that antisense-induced strain sensitivity profiles (AISS) generated by an extract of *Kibdelosporangium* sp. (MA7385), were consistent with those generated by the well-established coumarin antibiotic novobiocin.[82] Further analysis resulted in the isolation of the major active compound kibdelomycin (**79**) and its demethylated congener kibdelomycin A (**80**).[82] Kibdelomycin exhibited broad spectrum antibacterial activity against Gram-positive and some Gram-negative pathogens, including methicillin-resistant *S. aureus* (MIC = 0.5 μg mL^{-1}), and **79** was found to exert much more potent antibacterial activity against Gram-positive bacteria relative to Gram-negative bacteria.[82] However, this level of activity was significantly reduced (MIC = 64 mg mL^{-1}) when the compound was tested in the presence of 50% human serum. In contrast to kibdelomycin, **80** is less active against *S. aureus* (MIC = 2 μg mL^{-1}), suggesting that the methyl group at C-44 plays some important role for antibacterial activity.[82,83] Structure–activity relationship (SAR) studies for **79**, **80** and related congeners **81**, and **82** revealed that the methyl group at C-44, hydroxyl group at C-33, unsaturated olefin and chloride moieties play important roles in dictating antibacterial activity.[83] AISS profiles and biochemical enzyme assays suggested that **79** is the first novel class of bacterial type II topoisomerase inhibitors.[82] The likely mechanism of action for **79** involves inhibition of the ATPase activity of DNA gyrase and topoisomerase IV.[82] Compound **79** was found to show no cross-resistance to known bacterial type II topoisomerase inhibitors coumermycin A1, novobiocin and ciprofloxacin, indicating that **79** employs a unique binding mode in its interaction with DNA gyrase and topoIV. Compound **79** more potently inhibits *S. aureus* gyrase supercoiling (IC_{50} = 9 nM) and topoIV decatenation activity (IC_{50} = 500 nM) relative to *E. coli* gyrase supercoiling (IC_{50} = 60 nM) and topoIV decatenation activity (IC_{50} = 29 μM), and this finding is consistent with the results of antibacterial experiments.[82] *S. aureus* has a low frequency of resistance (FOR) against **79**; at 4- and 8-fold concentrations on the plate MIC, the FOR for **79** was $<5 \times 10^{-10}$ cfu mL^{-1}.[82,83] Interestingly, **80**, which differs from **79** by lacking the pyrrolic methyl appendage, was found to inhibit *S. aureus* gyrase supercoiling and topoisomerase IV decatenation activities with IC_{50} values of 400 and 5000 nM, respectively in stark contrast to the IC_{50}s noted for **79**.

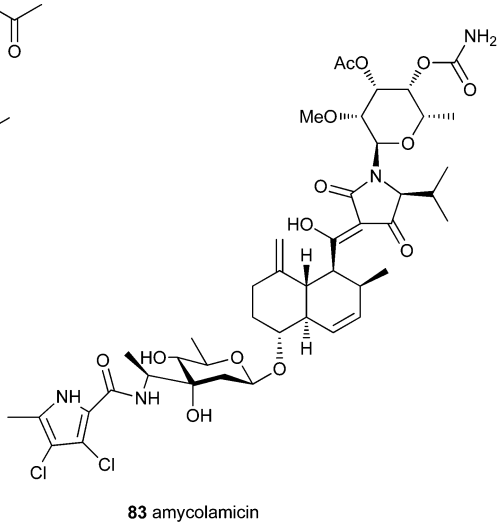
Sawa *et al.* discovered amycolamicin (AMM, **83**), akibdelomycin A analogue, from the broth of a soil actinomycete *Amycolatopsis* sp. MK575-fF5.[84] AMM is structurally composed of a *trans*-decalin, tetramic acid, two unusual sugars (amycolose and amykitanose), and a dichloropyrrole carboxylic acid. By combining a series of methods such as NMR spectroscopy, chemical degradation, X-ray analysis, and semi-synthesis, the chemical structure and stereochemistry of **83** were determined.[84] Additionally, the results of ^{13}C-labeled pyruvate feeding experiments demonstrated that pyruvate is the precursor of the branched α-aminoethyl moiety of amycolose.[85] Similar to kibdelomycin, **83** also showed broad spectrum potent antibacterial activity against Gram-positive bacteria and some Gram-negative bacteria as well as some drug resistant strains, including *S. aureus* (MIC = 0.125–2 μg mL^{-1}), methicillin-resistant *S. aureus* (MIC = 0.125–1 μg mL^{-1}), quinolone-resistant *S. aureus* (MIC = 0.25–1 μg mL^{-1}), *E. faecalis* (MIC = 0.25 μg mL^{-1}), vancomycin-resistant *E. faecalis* (MIC = 0.25 μg mL^{-1}), *E. faecium* (MIC = 1.0 μg mL^{-1}), vancomycin-resistant *E. faecium* (MIC = 0.5 μg mL^{-1}), *S. pneumoniae* (MIC = 0.25–0.5 μg mL^{-1}),



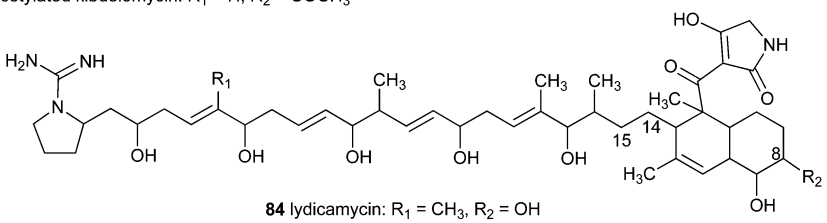
79 kibdelomycin: $R_1 = CH_3$, $R_2 = H$
80 kibdelomycin A: $R_1 = H$, $R_2 = H$
81 acetylated kibdelomycin: $R_1 = H$, $R_2 = COCH_3$



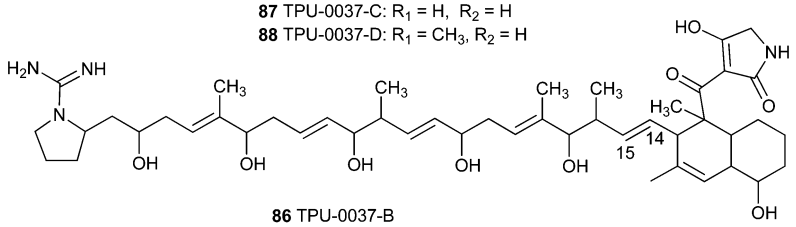
82 tetrahydro bis-dechloro kibdelomycin



83 amycolamicin



84 lydicamycin: $R_1 = CH_3$, $R_2 = OH$
85 TPU-0037-A: $R_1 = H$, $R_2 = OH$
87 TPU-0037-C: $R_1 = H$, $R_2 = H$
88 TPU-0037-D: $R_1 = CH_3$, $R_2 = H$



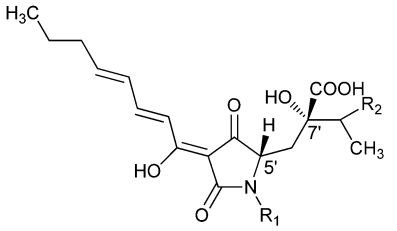
86 TPU-0037-B

penicillin-resistant *S. pneumoniae* (MIC = 0.25–0.5 μg mL^{-1}), *H. influenza* (MIC = 0.5–2.0 μg mL^{-1}), drug-resistant *H. influenza* BLNAR (MIC = 0.5–1.0 μg mL^{-1}) and *H. influenza* BLPACR (MIC = 1.0 μg mL^{-1}).[84] Similar to kibdelomycin, AMM is also a specific inhibitor of bacterial type II topoisomerase; it inhibited *E. coli* DNA gyrase (IC_{50} = 24.4 ng mL^{-1}) and topoisomerase IV (IC_{50} = 6250 ng mL^{-1}), coordinatively inhibiting DNA synthesis. Compound **83** failed to inhibit human type II topoisomerase (even at a dose of 25 μg mL^{-1}).[84] Notably, sequence analysis of DNA gyrase (GyrA and B subunits) and TopoIV (ParC and E subunits) from strains resistant to **83** indicated that its molecular target is the DNA gyrase B subunit and that **83** has a different binding mode than coumarin and quinolone antibiotics.[84]

Furumai *et al.* discovered four lydicamycin (**84**) congeners TPU-0037 A–D (**85–88**) from marine-derived *Streptomyces platensis* TP-A0598 isolated from a seawater sample collected in Toyama Bay, Japan.[86] The structures of **85–88** were determined on the basis of multiple spectroscopic data sets as well as by comparisons with **84**. Similar to **84**, **85–88** showed activity against Gram-positive bacteria including *S. aureus* and its MRSA strains, *B. subtilis* and *Micrococcus luteus*, with MIC values in the range of 0.39–12.5 μg mL^{-1}.[86] However, these compounds were found to be inactive against Gram-negative bacteria. Among **84–88**, compound **87** was found to display the most potent activity, and **86** displayed the lowest levels of activity, suggesting that the C14–C15 olefin may diminish antibacterial activity of these natural products.[86]

3.3 Dienoyltetramic acids

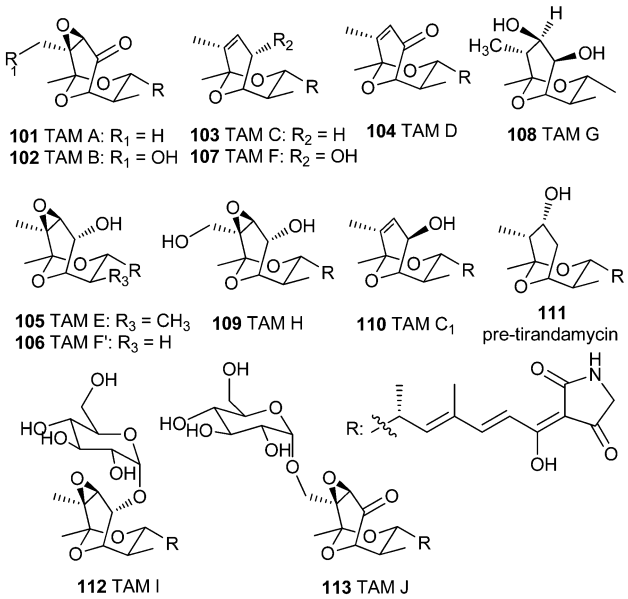
Harzianic acid (**89**) was first discovered from the culture filtrate of *Trichoderma harzianum* SY-307 isolated from a water sample collected at Hiroshima Pref. Japan in 1994.[87] In 2004, **89** was co-



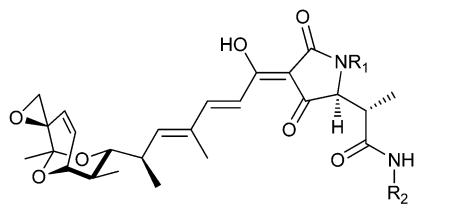
89 harzianic acid: $R_1 = CH_3$, $R_2 = CH_3$
90 demethylharzianic acid: $R_1 = H$, $R_2 = CH_3$
91 homoharzianic acid: $R_1 = CH_3$, $R_2 = CH_2CH_3$

isolated with demethylharzianic acid (**90**) and homoharzianic acid (**91**), from broths of the fungal strain F-1531 during the course of screens intended to identify novel serine/threonine phosphatase type 2A (PP2A) inhibitors.[7] In 2009, **89** was re-isolated from the EtOAc extracts of *T. harzianum* originating from composted hardwood bark in Western Australia.[88] Compound **89** was found to display antimicrobial activity against phytopathogenic *P. irregular*, *Sclerotinia sclerotiorum* and *Rhizoctonia solani*.[87,88] Harzianic acid is known to inhibit PP2A with an IC_{50} of 10 μg mL^{-1} in the active form which is composed of a complex of an **89**–Zn^{2+} complex in which two molecules of **89** bind one Zn^{2+} ion. In the absence of Zn^{2+}, complete loss of PP2A inhibitory activity by **89** has been noted.[7] All three compounds **89**, **90** and **91** exert weak cytotoxicity against human prostate cancer DU-145 cells with IC_{50}s of 17, 25, and 10 μg mL^{-1}, respectively.[7] Furthermore, **89** is a siderophore with good binding affinity for Fe^{3+} ($K_d = 1.79 \times 10^{-25}$), and this agents appears to play a role in plant growth regulation at low concentrations (<100 μM). However, high concentrations of **89** lead to inhibited seedling growth.[88,89]

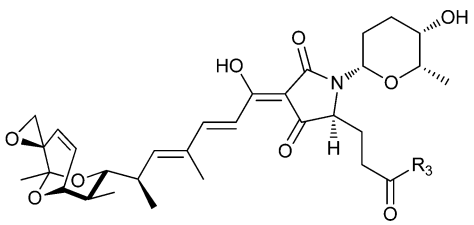
Streptolydigin (**92**), featuring two principal structural units: tetramic acid and bicyclic ketal structure, was isolated from culture filtrates of *Streptomyces lydicus* in 1955.[90] In recent years, by manipulating the streptolydigin biosynthesis gene cluster, several congeners of streptolydigin (**93–100**) were obtained in gene-engineered strains of *S. lydicus*.[91–93] Recent efforts aimed at understanding this class of compounds have also included the total synthesis of **92** as achieved by the Kozmin group.[94,95]



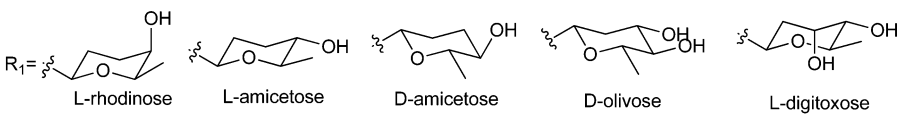
101 TAM A: $R_1 = H$
102 TAM B: $R_1 = OH$
103 TAM C: $R_2 = H$
107 TAM F: $R_2 = OH$
104 TAM D
108 TAM G
105 TAM E: $R_3 = CH_3$
106 TAM F': $R_3 = H$
109 TAM H
110 TAM C_1
111 pre-tirandamycin
112 TAM I
113 TAM J



92 streptolydigin: R_1 = L-rhodinose, $R_2 = CH_3$
93 demethyl-streptolydiginone: $R_1 = H$, $R_2 = H$
94 streptolydiginone: $R_1 = H$, $R_2 = CH_3$
95 streptolydigin LA: R_1 = L-amicetose, $R_2 = CH_3$
96 streptolydigin DO: R_1 = D-olivose, $R_2 = CH_3$
97 streptolydigin DA: R_1 = D-amicetose, $R_2 = CH_3$
98 streptolydigin LD: R_1 = L- digitoxose, $R_2 = CH_3$



99 streptolydigin B: R_3=OH
100 streptolydigin C: R_3= $NHCH_3$



L-rhodinose L-amicetose D-amicetose D-olivose L-digitoxose

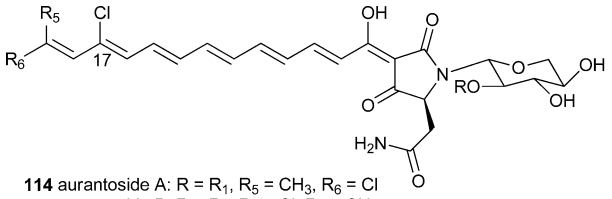
Streptolydigin is an inhibitor of bacterial RNA polymerase (RNAP), interfering with RNA chain elongation during transcription. The co-crystal complex of **92** with the *Thermus thermophilus* RNAP and *E. coli* RNAP revealed the mechanism and structural basis of RNAP inhibition.[96,97] Streptolydigin stabilizes the straight-bridge-helix active-center conformation and blocks the structural isomerization required for RNAP function by binding to the RNAP active center.[96,97] Early results revealed that **92** shows activity against a number of Gram-positive bacteria and that its antibacterial activity is generally more potent than that of tirandamycin A and tirandalydigin.[1] Bioassays with *S. albus* using a disk-diffusion method revealed that streptolydiginone, streptolydigin LA, streptolydigin B are less active than **92**, whereas, streptolydigin C shows slightly greater activity than **92**.[91–93] Furthermore, lower levels of antibacterial activity against *Streptococcus salivarius* for streptolydiginone and synthetic analogs have been observed.[95] These results indicate that the bicyclic ketal and tetramic acid units of these agents play important roles in setting streptolydigin activity and that the deoxyhexose moiety appears to improve antibacterial activity.

Tirandamycins (TAMs) **101–113** are structural analogs of streptolydigin and are isolated from a series of marine and terrestrial *Streptomyces* species and their mutant strains. Tirandamycin A (**101**) and B (**102**) were initially isolated from *Streptomyces tirandis* and *S. flaveolus* in the 1970s.[98,99] In 2009, tirandamycins C (**103**) and D (**104**) along with **101** and **102**, were isolated as intermediates from the broth of marine-derived *Streptomyces* sp. 307-9 using a resin-based metabolite-trapping method.[100] Almost at the same time, our group discovered **101** and **102** from the broth of *Streptomyces* sp. SCSIO1666 obtained from sediments of the South China Sea.[101] In searching for drug leads targeting asparaginyl-tRNA synthetase of *Brugia malayi*, the causative agent of lymphatic filariasis, Yu *et al.* discovered tirandamycins E (**105**), F′ (**106**) and G (**108**) along with tirandamycins A and B from *Streptomyces* sp. 17944 in 2011.[102] Tirandamycins H–J (**109**, **112**, **113**) were subsequently isolated from the same strain following the application of medium optimization methods.[103] Espinosa and co-workers re-isolated tirandamycin A from the marine-derived *Streptomyces* sp. URI-F11 originating from sediment samples collected from Fishers Island Sound, NY.[104] Meanwhile, tirandamycins C, E, F (**107**), C1 (**110**) and pre-tirandamycin (**111**) were isolated from genetically engineered strains of *Streptomyces* sp. 307-9 and *Streptomyces* sp. SCSIO1666.[105–109] X-ray crystallography using the *p*-bromophenacyl ester of tirandamycic acid enabled elucidation of tirandamycin A absolute configuration.[110] Interestingly, the total synthesis of tirandamycin A was achieved by several groups in the mid 1980s, whereas the total synthesis of tirandamycin B was first accomplished in 1991.[111,112] More recently, the total synthesis of tirandamycin C was achieved.[113,114] Similar to streptolydigin, tirandamycin A is an inhibitor of RNA polymerase interfering with transcription initialization and elongation although its activity in this manifold is inferior to streptolydigin by ~40 fold.[115] The tirandamycins display numerous activities and the C-18 hydroxyl group along with different oxidative modifications in the bicyclic ketal, are essential to tirandamycin activity. Tirandamycin A (MIC = 2.25 μM) has been found to display potent activity against vancomycin-resistant *E. faecalis*, followed by is tirandamycin D (MIC > 9 μM), then tirandamycin B (MIC = 100 μM), and tirandamycin C (MIC = 110 μM).[100] Of the tirandamycins, tirandamycin B exhibits the highest inhibitory activity against *Brugia malayi* asparaginyl-tRNA synthetase (AsnRS) displaying an IC_{50} of 30 μM. Against the same enzyme, the other seven compounds (tirandamycins A, E–J) display significantly reduced activity (IC_{50} > 200 μM).[102,103] Moreover, the selectivity of tirandamycin B for the *B. malayi* AsnRS is minimally 10-fold over the human AsnRS.[102] Furthermore, tirandamycin B, by virtue of its AsnRS inhibitory activity, has been shown to efficiently kill adult *B. malayi* worms (the causative agent in River Blindness Disease) *in vitro* with an IC_{50} of 1 μM.[102] These findings suggest that tirandamycin B represents a promising lead scaffold able to be exploited as antifilarial drug. In addition, at a concentration of 60 μM, tirandamycin A has been shown to inhibit the causative agents for amoebic dysentery *E. histolytica* HM-1:IMSS (84.2% inhibitory rate) and a clinical isolate *E. histolytica* Col (64.8% inhibitory rate).[104]

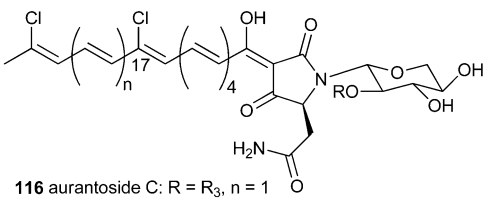
3.4 Polyenoyltetramic acids

The aurantosides are a series of orange-red pigments isolated from marine sponges featuring three different structural units: a mono- or dichlorinated and long (C18 to C24) conjugated polyene chain, a tetramate ring, and an *N*-glycosidic moiety composed of one to three monosaccharide units. Before 2002, six compounds of this family aurantosides A–F (**114–119**) were isolated from sponge *Theonella* sp., *Homophymia conferta* and *Siliquaria spongia japonica*, respectively.[116–118] In 2005, three new members aurantosides G–I (**120–122**) were isolated from lithistid sponge *Theonella swinhoei* collected from Papua New Guinea and Bunaken Marine Park of Manado (North Sulawesi, Indonesia), respectively.[119] In 2011, aurantosides G–I, along with the new member aurantoside J (**123**), were re-isolated from *T. swinhoei* collected from the same area previously associated with **120–122**.[120] The latest member aurantoside K (**124**), together with **122** and **114**, was obtained from MeOH extracts of *Melophlus* sp. sponge collected from the Fiji Islands in 2012.[121] To date, all members of the aurantosides have been isolated from marine sponges and are derived from L-aspartic acid and are decorated with D-saccharides. These realizations may well be indicative of a common biosynthetic pathway shared among these polyene tetramic acid metabolites. The sugar attached to the tetramic acid *N*-moiety of aurantoside J was determined to be *N*-α-xylopyranose,[120] and all other aurantosides have been assigned as *N*-β-glycosidic linkages.

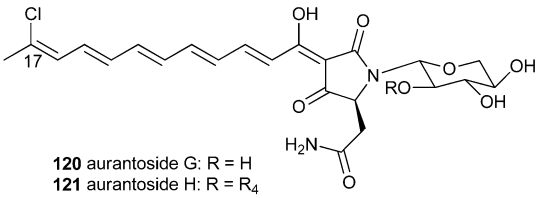
Compounds **120–122** and **124** failed to show significant cytotoxicity activity.[119–121] The cytotoxicity data for the aurantoside family that is available in the literature clearly suggest a concrete structure–activity relationship, most prominently, an increase in polyene chain length is associated with improved cytotoxicity. Accordingly, **120–124** (C18) are not cytotoxic, **114** and **115** (C20) are weakly cytotoxic compared to congeners **117** and **118** (C22), whereas **119** (C24), bearing the longest polyene,



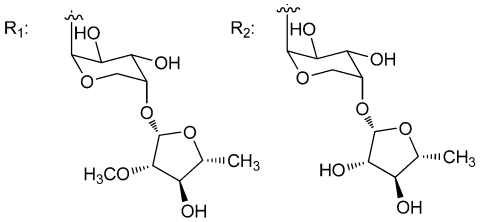
114 aurantoside A: R = R_1, R_5 = CH_3, R_6 = Cl
115 aurantoside B: R = R_2, R_5 = Cl, R_6 = CH_3

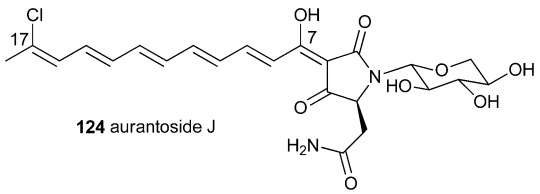


116 aurantoside C: R = R_3, n = 1
117 aurantoside D: R = R_2, n = 1
118 aurantoside E: R = R_1, n = 1
119 aurantoside F: R = R_1, n = 2

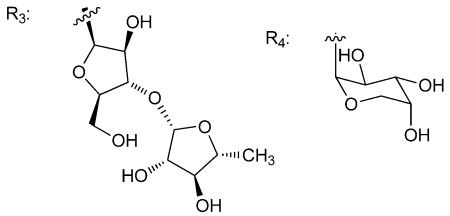


120 aurantoside G: R = H
121 aurantoside H: R = R_4
122 aurantoside I: R = R_1
123 aurantoside K: R = R_2



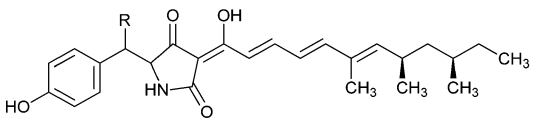


124 aurantoside J

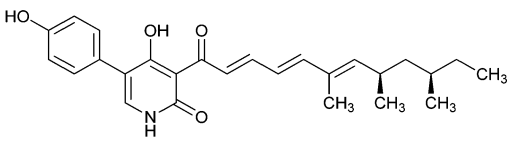


displays the most pronounced cytotoxicity of the structural class. In addition, **120** displayed moderate activity against *Candida* sp. strains, **122** and **124** displayed appreciable antifungal activity against a variety of fungus.[119–121] However, **121** and **123** were found to be completely devoid of antifungal activity.[119–121]

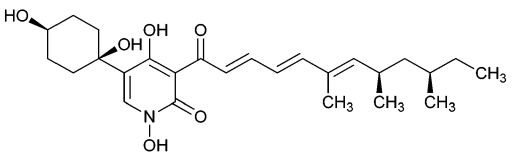
Two tetramic acid compounds, militarinones B (**125**) and C (**126**), together with pyridinone-based militarinone D (**127**), were isolated from the methanolic mycelial extracts of the entomopathogenic fungus *Paecilomyces militaris* RCEF 0095 in 2003.[122] NMR spectra indicated that **125** and **126** exist as mixtures of two tautomers, namely, the *exo*- and *endo*-forms,



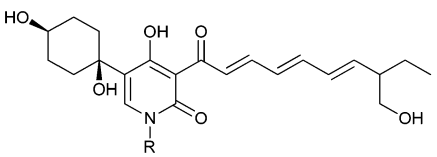
125 militarinone B: R = OH
126 militarinone C: R = H



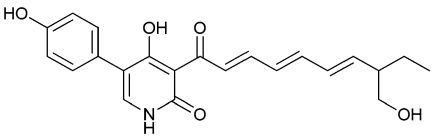
127 militarinone D



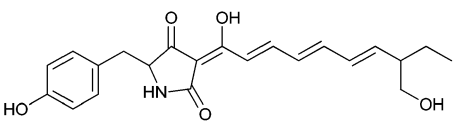
128 militarinone A



129 torrubiellones A: R = OH
130 torrubiellones B: R = H



131 torrubiellones C



132 torrubiellones D

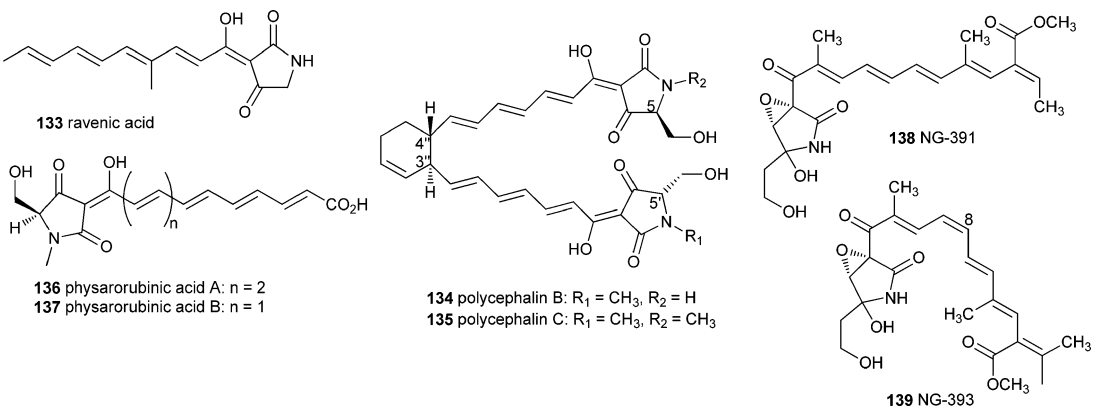
although the precise tautomer ratios remain unclear.[122] Compounds **125** and **126** showed negligible neuritogenic activity in PC-12 cells at 100 μM and **127** was found to be completely inactive at this concentration.[122] The absence of neuritogenic activity by **125–127** is interesting given that it was the observation of distinct neuritogenic activity by components of the mycelial extract of *P. militaris* RCEF 0095 that served as the original catalyst for discovery of these compounds. Ultimately, activity-directed fractionations of the same extract identified militarinone A (**128**), a potent neuritogenic agent.[123] Assessments of cytotoxicity in PC-12 cells using lactate dehydrogenase assays revealed that **127** exerts significant cytotoxicity at 100 μM (74.0%) and 33 μM (30.7%); the same assays revealed that **125** is also active as well (16.8% at 100 μM) and that **126** is inactive.[122]

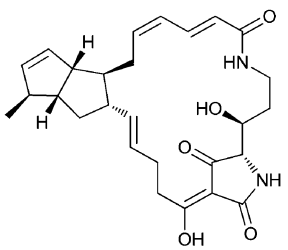
The tetramic acid torrubiellone D (**132**) along with three pyridine compounds torrubiellones A–C (**129–131**) were identified from extracts of *Torrubiella* sp. BCC 2165 in 2010.[124] Similar to closely related **126**, ^{1}H NMR spectra revealed that **132** exists as a 5 : 1 mixture of *exo*-enol tautomers. Compound **132** was only found to exhibit weak cytotoxicity against KB cell line (IC_{50} = 44 μM).[124] The identification of **125–128** and **129–132** provides support for the hypothesis that pyridine alkaloid biosynthesis proceeds through an acyl tetramic acid intermediate. Further support for this hypothesis is provided by the realization that pyridine alkaloid compounds **128** and **129** are major metabolites.[123,124]

Ravenic acid (**133**), isolated from EtOH extracts of the mycelium from *Penicillium* sp. (MINAP9902), exists as the *exo*-enol tautomer and is present as an equilibrating mixture of 3*Z*/3*E* double-bond isomers in a 4 : 1 ratio.[125] The first total synthesis of **133** was achieved by Schlenk and co-workers in 2010.[126] Compound **133** was found to display activity against methicillin-resistant *S. aureus*, *M. phlei* and *S. aureus*.[125,126] However, **133** failed to display significant biological activity against *E. colitolC*, *K. pneumonia* and *M. luteus*.[126] Furthermore, **133** had little effect on human umbilical vein endothelial cells and erythrocyte membranes with ED_{50} and IC_{50} values of >100 μm.[126] Bioassays with synthetic analogs of **133**, have revealed that the high degree of unsaturation of the C-3 side chain is not required for antimicrobial or cytotoxic activity.[126]

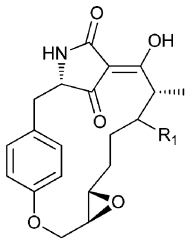
Nowak and Steffan discovered polycephalin B (**134**) and C (**135**) along with physarorubinic acid A (**136**) and B (**137**) from the myxomycetes culture of *Physarum polycephalum*, and found that the yields of **134** and **135** are highly dependent on light.[127] As **134** and **135** are sensitive to light, all procedures from incubation to extraction were conducted in the absence of light and at low temperature. The *S* configuration at the C-5 and C-5′ positions of the tetramic acid moiety has been established on the basis of hydration experiments and comparisons of the CD spectra with the hydration products to those obtained using synthetically generated 3-acetyl-5-hydroxymethyl-*N*-methyltetramic acid.[128] The 3″*R* and 4″*R* stereochemical configurations were proposed following comparisons of CD spectra for polycephalin C and synthetic compounds; ultimately these assignments were confirmed by the total synthesis of polycephalin C.[128–130]

The neuronal cell-protecting molecule NG-391 (**138**) and its 8*Z* stereoisomer NG-393 (**139**) were first isolated from an unidentified *Fusarium* sp. TF-0452 in 1996.[131] Compound **138** was later re-isolated from *Fusarium* sp. RK97-94 in 2001.[132] During screens to detect destruxin production by mutants derived from the entomopathogenic fungus *Metarhizium anisopliae* ARSEF 2575, Gibson and co-workers reported isolation of **138** and **139** from the mutant strain KOB1-3 in 2006.[133] The structure of **138** was determined on the basis of extensive spectroscopic data and the absolute configuration ultimately assigned by the application of total synthesis.[131,132,134] Compound **138** was found to show neurotrophic activity, although no antimicrobial or insecticidal activities could be detected.[131,133] Compound **139** was also found to be inactive against bacteria and fungi.[133] Conversely, **138** and **139** were found to display potent S9-dependent mutagenic activities with significant dose–response affects during Ames assays.[133] This latter activity has raised concerns about the potential risks associated with the use of *Metarhizium anisopliae* as a biocontrol agent.

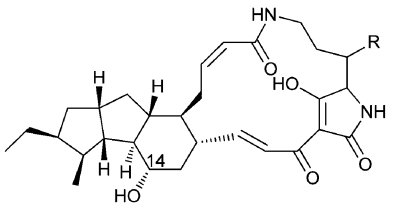




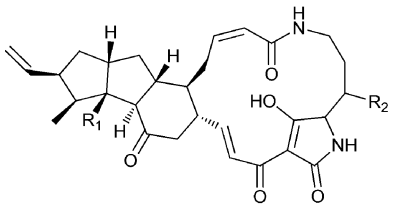
140 cylindramide A



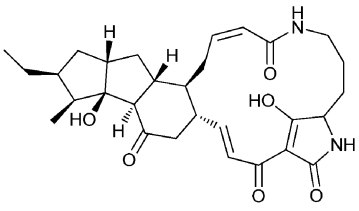
141 macrocidin A: R_1 = H
142 macrocidin B: R_1 = OH



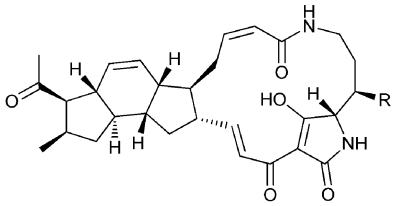
143 HSAF: R = OH
144 3-deOH HSAF: R = H



145 frontalamide A: R_1 = OH, R_2 = OH
146 frontalamide B: R_1 = H, R_2 = OH
147 frontalamide FI-1: R_1 = OH, R_2 = H
149 frontalamide FI-3: R_1 = H, R_2 = H



148 frontalamide FI-2



150 clifednamides A: R = H
151 clifednamides B: R = OH

3.5 Macrocyclic tetramic acids

Cylindramide A (**140**) was isolated from MeOH extracts of the sponge *Halichondria cylindrata* collected off the Sata Peninsula, Shikoku and its stereochemistry elucidated on the basis of total synthesis efforts.[135–139] Compound **140** has shown potent cytotoxic activities against cancer cells B16 melanoma, L929, L-R118, PtK_2, A-431, PC-3S, KOV-3, KB-3-1, verapamil-resistant KB-V1, K-562, U-397 with IC_{50}s in the range from 0.067 μg mL^{-1} to 0.8 μg mL^{-1}.[135,139] In addition, it is evident that **140** exhibits more potent cytotoxicity than its synthetic analogs suggesting that the tetramic acid moiety might be more important for activity than the correct stereochemistry at C-2.[139] Furthermore, the bicyclo[3.3.0]octene moiety appears to be essential for activity. Ca^{2+} was found to have a significant effect on the activity of **140** against L929 and U-937, whereas Mg^{2+} had no effect on either cell line, suggesting that Ca^{2+}–ligand complexes may be the biologically active species or that bioactivity is somehow dependent on the formation of such complexes *in situ*.[139]

Macrocidins A (**141**) and B (**142**), were isolated from liquid cultures of *Phoma macrostoma* collected from diseased Canada thistle, and the relative stereochemistry and absolute configuration of **141** were deciphered on the basis of spectroscopic analyses, single-crystal X-ray diffraction and total synthesis.[140–142] Macrocidins provide the first example of a naturally occurring acyltetramic acid within large rings containing a tyrosine amino acid; rotation of the aromatic ring appears to be somewhat restricted as inferred from line broadening of the NMR.[140] The biological activities of **141** and **142** have been assayed using greenhouse-grown plants including sunflower (*Helianthus annuu*), giant foxtail (*Setaria faberi*), ivyleaf morning glory (*Ipomoea hederaceae*), wild oat (*Avena fatua*), a barnyard grass (*Echinochloa crusgalli*). Notably, significant chlorosis and growth inhibitory activities were expressed by **141** and **142** against broadleaf weeds but not grass weeds.[140] Although whole plant symptoms caused by the macrocidins are similar to those induced by sulcotrione and hydroxyphenyl pyruvate dioxygenase (HPPD) inhibitors, no significant inhibition of HPPD with macrocidins was observed *in vitro*.[140] Consequently, the mode of action for these compounds remains unknown. It is worth mentioning that bleaching and stunting appeared primarily in newly grown susceptible weeds, suggesting these compounds were phloem mobile.[140]

HSAF (heat-stable antifungal factor, **143**) was produced by *Lysobacter enzymogenes* C3 strain which was isolated from grass foliage.[143,144] *L. enzymogenes* C3 has been used to control plant fungal diseases in agriculture and it is known to inhibit multiple fungal pathogens such as *Rhizoctonia solani* and *Bipolaris sorokiniana*.[143,145–148] Subsequently, 3-deOH HSAF (**144**) was isolated from a mutant producer lacking sterol desaturase gene.[144,149] HSAF displayed potent activity against a broad range of fungi and was found to be inactive against assorted bacteria.[150] The effect of HSAF on fungal growth manifests in a concentration dependent manner; HSAF inhibited spore germination under higher concentration (40 μg mL^{-1}), whereas, at low concentrations, it suppressed hyphal elongation and induced excessive branching (20 μg mL^{-1}).[150] Notably, HSAF appears to exert a new mode of action by disrupting the biosynthesis of fungal sphingolipids and blocking fungal-polarized growth.[151] In contrast to HSAF, 3-deOH HSAF (**144**) failed to exert antifungal activity against *Penicillium avellaneum*,

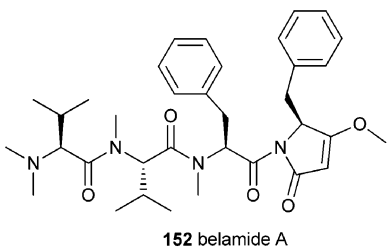
suggesting the importance of the 3-hydroxyl group for antifungal activity.[149]

Frontalamides A (**145**) and B (**146**) were discovered from a symbiotic bacterium of the southern pine beetle (*Dendroctonus frontalis*) *Streptomyces* sp. SPB78.[152] By manipulating the biosynthetic gene cluster, three frontalamide analogs FI-1, FI-2, FI-3 (**147–149**) were produced using an engineered strain of *Streptomyces* sp. SPB78 *ΔftdA* which lacks the gene encoding alkane hydroxylase.[152] Frontalamides A and B were found to exert antifungal activity against *Ophiostoma minus*. In contrast, **147–149** showed no activity against *O. minus*.[152] This finding implies that the C-3 hydroxyl group within the frontalamide scaffold is essential for antifungal activity.

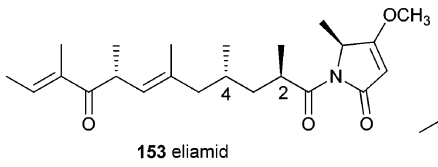
Based on the conserved arrangement of *ftdA–ftdB* within the biosynthetic gene cluster of polycyclic tetramate macrolactams, a degenerate primer was designed to probe environmental actinomycete isolates bearing frontalamide-like biosynthetic genes. This PCR-guided strategy led to the discovery of clifednamides A (**150**) and B (**151**) bearing the unusual 5-6-5 set of carbocyclic rings from the PCR positive strain *Streptomyces* sp. JV178.[153] The C-25 hydroxyl group in **151** was determined to be in the β-orientation based on molecular modeling. This assignment was further supported by the well known and common biosynthetic mechanism of L-ornithine incorporation calling for the homologous protein alkane hydroxylase FtdA.[153] Importantly, members of both the HSAF frontalamide compound classes display interesting polycyclic structures whose modes of installation remain somewhat speculative and a topic for discussion and further study. The precise means by which the polycyclic scaffold of these compounds undergoes construction clearly represents a target for future studies.

3.6 *N*-Acyl-4-methoxy-3-pyrrolin-2-ones

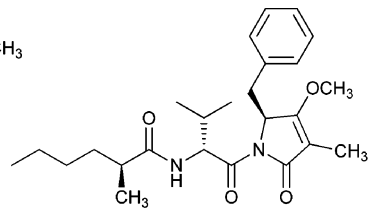
Belamide A (**152**), the highly methylated linear tetrapeptide analogue of dolastatins 10 and 15, was discovered from the Panamanian marine cyanobacterium *Symploca* sp.[154] All amino acids of **150** were assigned as L-configurations following hydrolysate analysis with chiral HPLC and these stereochemical



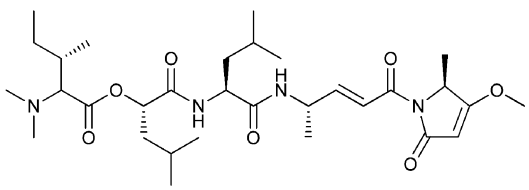
152 belamide A



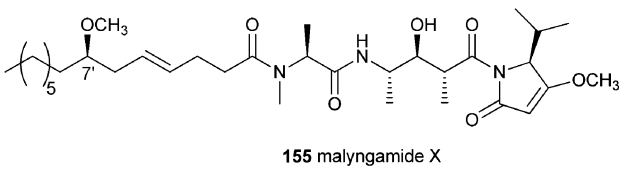
153 eliamid



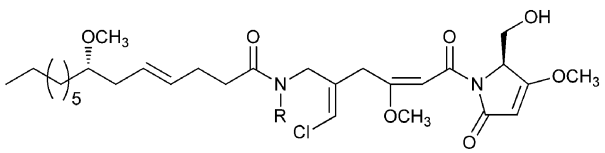
164 palau'imide



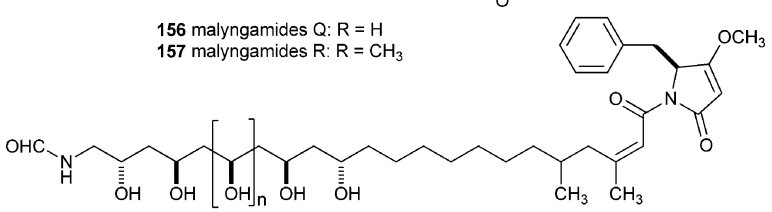
154 symplostatin 4 (gallinamide A)



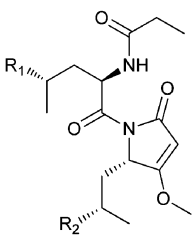
155 malyngamide X



156 malyngamides Q: R = H
157 malyngamides R: R = CH_3



158 mycapolyol A (n = 10)
159 mycapolyol B (n = 9)
160 mycapolyol C (n = 8)
161 mycapolyol D (n = 7)
162 mycapolyol E (n = 6)
163 mycapolyol F (n = 5)



165 sintokamide A: $R_1 = CCl_3$, $R_2 = CHCl_2$
166 sintokamide B: $R_1 = CCl_3$, $R_2 = CCl_3$
167 sintokamide C: $R_1 = CHCl_2$, $R_2 = CHCl_2$
168 sintokamide D: $R_1 = CCl_3$, $R_2 = CH_2Cl$
169 sintokamide E: $R_1 = CCl_3$, $R_2 = CH_3$

assignments were later validated by asymmetric total synthesis of **152**.[154,155] Compound **152** was found to be cytotoxic to MCF7 and HCT-116 cell lines with IC_{50} values of 1.6 μM and 0.74 μM, respectively. In addition, **152** showed classic microtubule depolymerizing effects toward A-10 cells at 20 μM.[154]

Eliamid (**153**) was isolated from two strains: ambruticins producer *Sorangium cellulosum* So ce439 (DSMZ 11529) and soraphen producer *S. cellulosum* So ce241.[156] The relative configurations of the C-2 and C-4 methyl groups were assigned as *anti* on the basis of Breit's rule and the absolute configuration of all stereocenters was determined by a combination of degradation methods, structural similarity analysis and total synthesis.[156] Notably, Breit's rule dictates that by using methylene proton NMR signal assignments and determination of the chemical shift differences ($\Delta\delta$), the relative configuration of 1,3,*n*-methyl-branched deoxypropionates can be determined directly.[157] Eliamid was found to exert potent activity against a panel of transformed cell lines with the IC_{50} values in the range 0.5–30.0 ng mL^{-1}.[156] In addition, **153** showed cytotoxic activity against brine shrimp *Artemia salina* at 0.2–0.3 μg mL^{-1}, lethal activity against soil nematodes (*Panagrellus* spec.) at 5 μg mL^{-1}, and moderate inhibitory activity against fungi and yeast in agar diffusion assays (ranging from 15–22 mm zone at 20 μg per disk).[156] The mode of action was determined by examining the effects of **153** on the mitochondrial respiratory chain. Compound **153** was found to strongly inhibit NADH oxidation in beef heart submitochondrial particles (SMP) with an IC_{50} of 8 ng mL^{-1} and differential spectroscopy experiments with beef SMP indicated that **153** is a potent inhibitor of complex I (NADH-ubiquinone oxidoreductase) within the eukaryotic respiratory chain.[156]

Linington *et al.* isolated gallinamide A (**154**) from the extract of a *Schizothrix* sp. strain originating from a tropical reef near Piedras Gallinas by using bioassay-guided method.[158] At first, the planar chemical structure of **154** was determined by extensive NMR analyses, whereas absolute configurations at some asymmetric centers [except for *N*,*N*-dimethylisoleucyl (C-25 and C-26)] were assigned using a combination of degradation and derivatization methods.[158] In 2011, Conroy and co-workers achieved the total synthesis of **154** and discovered that the structures of gallinamide A and symplostatin 4 are identical.[159] Symplostatin 4 was isolated from the marine cyanobacterium *Symploca* sp. collected from Key Largo following the use of a cytotoxicity-guided fractionation method, and its absolute configuration determined by chiral HPLC analysis of its degradation products as well as total synthesis.[160,161] Compound **154** and three synthetically generated *N*-terminal diastereoisomers demonstrated potent antimalarial activities against *P. falciparum* 3D7 strain (IC_{50} = 37–104 nM), similar to that associated with the antimalarial chloroquine (IC_{50} = 17.8 nM), and **154** also showed moderate activity against a chloroquine-resistant strain of *Plasmodium falciparum* W2 (IC_{50} = 8.4 μM). It is worthy noting that **154** did not cause red blood cell lysis even at concentrations as high as 25 μm.[158,159,161] Unlike the dolastatins, which also possess antimalarial activity as well as significant cytotoxicity, **154** showed moderate cytotoxicity against mammalian Vero cells (TC_{50} = 10.4 μM), HeLa cervical carcinoma cells (IC_{50} = 12 μM) and HT-29 colon adenocarcinoma cells (IC_{50} = 53 μM). It also failed to show detectable activity against NCI-H460 human lung tumor and neuro-2a mouse neuroblastoma cell lines at the test concentration (16.9 μM), suggesting that **154** serves as a promising lead scaffold for antimalarial drug discovery.[158,160] Moreover, **154** can disrupt cellular microtubules at 50 μM, arresting the cell cycle at G2/M phase albeit with lower potency than the dolastatins. Notably, a synergistic effect between **154** and largazole, an inhibitor of histone deacetylases produced by **154**-producing *Symploca* sp. was observed upon simultaneous treatment of HT-29 cells with **154** and largazole.[160] Treatment of *P. falciparum*-infected red blood cells with symplostatin lead to generation of a food vacuole phenotype and inhibited pathogen replication with an EC_{50} of 0.7 μM. Further studies revealed that **154** is a nanomolar inhibitor of the *P. falciparum* falcipains, suggesting that its mode of action entails inhibition of the hemoglobin degradation pathway and that its unusual methylmethoxypyrrolinone group is essential for antimalarial activity.[162]

Suntornchashwej *et al.* isolated malyngamide X (**155**) from the sea hare *Bursatella leachii* collected from the Gulf of Thailand.[163] The structure and 2*S*, 5*S*, 7*S*, 8*R*, 14*S*, 7′*R* stereochemistry of **155** was determined using a combination of MS, NMR spectroscopic analyses, molecular mechanics calculations, NMR chiral solvation experiments and synthetic molecular fragment analyses, the conclusions of which were ultimately confirmed by total synthesis.[163,164] Compound **155** was the first (7*R*)-lyngbic acid found to be connected to a tripeptide backbone in contrast to all other members of the malyngamide class known at the time. Compound **155** exhibited moderate cytotoxicity against a series of cancer cell lines including oral human epidermoid carcinoma of the nasopharynx KB (ED_{50} = 8.20 μM), human small cell lung cancer NCI-H187 (ED_{50} = 4.12 μM), and breast cancer BC (ED_{50} = 7.03 μM).[163] It also was found to display antitubercular activities against *M. tuberculosis* strain H37Ra (MIC = 80 μM), and antimalarial activity against multidrug-resistant *Plasmodium falciparum* strain K1 (ED_{50} = 5.44 μM).[163]

Malyngamides Q (**156**) and R (**157**) were isolated from the lipid extract of Madagascan *Lyngbya majuscule* and their structures including stereochemistry were determined by analyzing multiple NMR spectroscopic as well as analyzing hydrolysis products using chiral GC-MS, which was also confirmed by total synthesis.[165,166] Compound **156** is not stable and readily decomposes; thus its biological activity was not evaluated. Compound **157** exhibited toxicity toward brine-shrimp (LD_{50} = 18 ppm).[165]

Phuwapraisirisan and co-workers obtained mycapolyols A–F (**158–163**) from the marine sponge *Mycale izuensis*.[167] The structures and stereochemistry of mycapolyol A–F were assigned on the basis of spectroscopic data analyses, comparisons with analogues and the application of Kishi's ^{13}C NMR database for the 1,3,5-polyol system. Compounds **158–163** were found to exert potent cytotoxicity against HeLa cells with IC_{50} values of 0.06, 0.05, 0.16, 0.40, 0.38, and 0.90 μg mL^{-1},

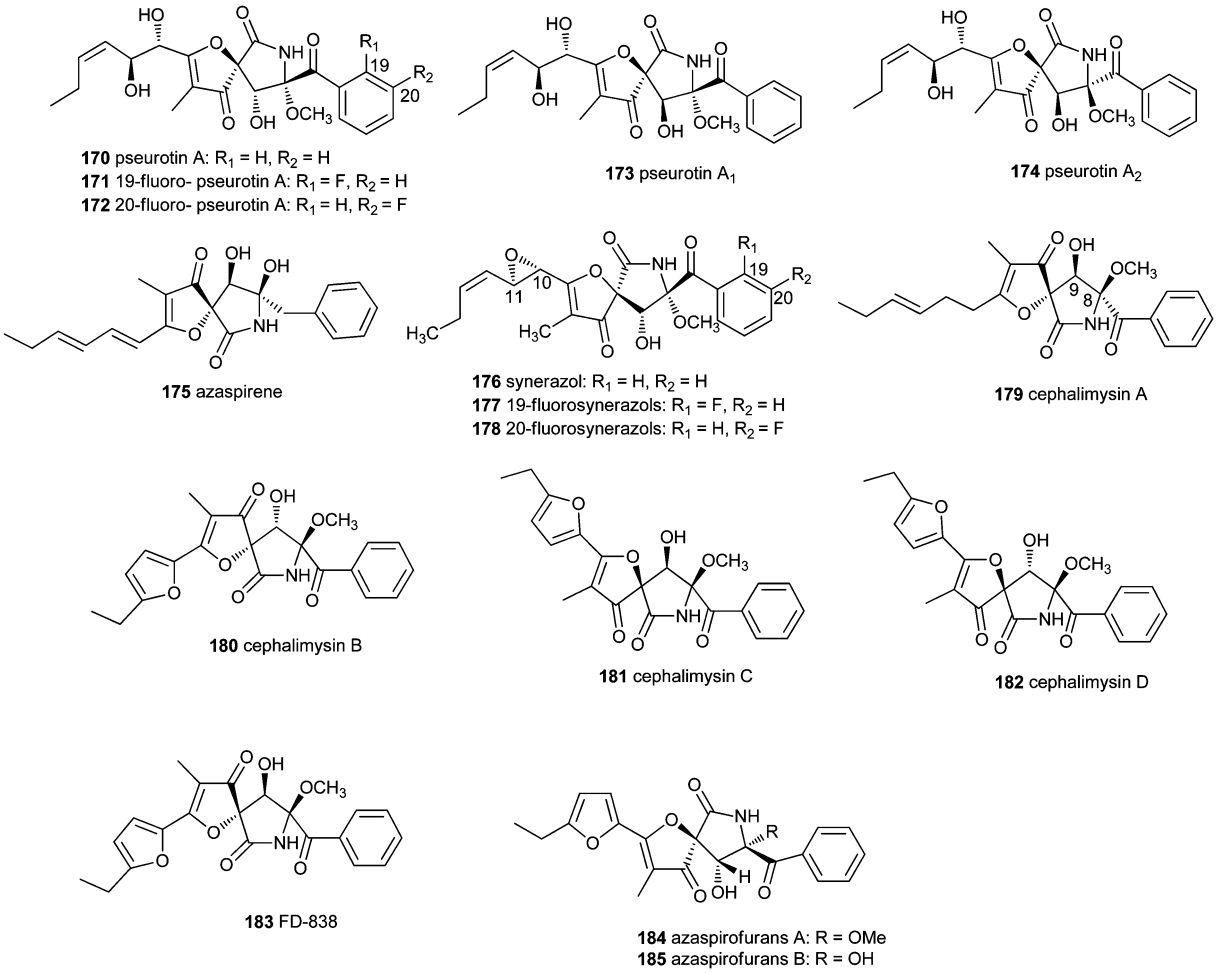
respectively, implying that the chain length of the 1,3-polyol system may be essential for activity.[167]

Luesch *et al.* discovered palau'imide (**164**) along with several other alkaloids from the apratoxin-producing marine cyanobacterium *Lyngbya* sp. collected in Palauan.[168] The configurations of C-4 and C-15 were determined by analyzing degradation products and the 20*S* stereochemistry was determined by total synthesis.[168–170] Palau'imide was found to display *in vitro* cytotoxicity against KB cells (IC_{50} = 1.4 μM) and LoVo cells (IC_{50} = 0.36 μM).[168]

During the course of screening marine natural product extracts for inhibitors of transactivation of the androgen receptor *N*-terminus domain in LNCaP human prostate cancer cells, Sadar and co-workers identified five chlorinated peptide compounds, sintokamides A–E (**165–169**) from MeOH extracts of the sponge *Dysidea* sp. collected in Indonesia.[171] The 2*S*, 4*S*, 10*R*, 16*S* configurations were assigned on the basis of X-ray diffraction data.[171] Sintokamides B–E differ from **165** in their degree of chlorination at C-18 or C-19. Sintokamide A is the first known inhibitor of androgen receptor N-terminus transactivation in prostate cancer cells showing an inhibitory effect at 5 μg mL^{-1}.[171]

3.7 3-Spirofuranone-lactam tetramic acid

Pseurotin A (**170**) was originally isolated from the broth of fungal strains *Pseudeurotium ovalis* Stolk and *A. fumigates* in 1976 and 1993.[172,173] It was later re-isolated along with two fluorinated analogs, 19- and 20-fluoro-pseurotin A (**171**, **172**), along with pseurotin A1 (**173**) and pseurotin A2 (**174**) from two different *A. fumigates* strains.[174,175] The absolute stereochemistry of **170** was determined by X-ray crystallographic analysis and confirmed by total synthesis.[176] Compound **170** and its fluorinated analogs displayed activity against *S. cerevisiae* (MICs = 50 μg mL^{-1}).[174] Compound **173** was inactive against P388, HL-60, A549, and BEL-7402 cancer cell lines, although **174** displayed slight cytotoxicity against A549 (IC_{50} = 40.8 μM) and HL-60 (IC_{50} = 70.8 μM) cells. Pseurotin A (**170**) was slightly cytotoxic to HL-



60 (IC_{50} = 67.0 μM) cells and it was found to inhibit IgE production with an IC_{50} of 3.6 μM.[175,177]

Azaspirene (**175**) was isolated from the culture of fungus *Neosartorya* sp. and its absolute structure was determined by total synthesis.[178,179] Compound **175** was found to completely inhibit human umbilical vein endothelial cell migration induced by vascular endothelial growth factor (VEGF) at 27.1 μM without any significant cell toxicity.[178] The results of *in vivo* assays revealed that **175** reduced the number of tumor-induced blood vessels through a mechanism that suppresses Raf-1 activation and subsequent activation of VEGF receptor-2. In addition, it was found that **175** does not inhibit non-vascular endothelial, NIH3T3, HeLa, MSS31, and MCF-7 cells.[180]

Synerazol (**176**), possesses an epoxy functionality at C-10/C-11 instead of the 1,2-dihydroxy group that characterizes **170**. Compound **176** was originally isolated from *Aspergillus fumigates* SANK10588, and was then re-isolated from *A. fumigates* TP-F0196 along with two novel fluorinated analogs, 19- and 20-fluorosynerazols (**177**, **178**) generated as a result of feeding experiments with fluorophenylalanine.[174,181] The absolute configuration of **176** was determined by application of a modified Mosher's method and confirmed by total synthesis.[182] Compound **176** as well as its fluorinated analogs exhibited antifugal activity against multiple fungi such as *Candida* spp., but failed to demonstrate any significant antibacterial activity although synergistic activities with azole-type antifungal agents was observed.[174,181] Cytotoxicities against a series of human tumor cell lines were noted; IC_{50} values spanned the range from 0.09–86 μM.[181] Chorioallantoic membrane assays demonstrated that **177** and **178** exert significant inhibitory effects on neovascularization in a dose-dependent manner.[181] Additionally, **176** showed significant inhibitory activities against IgE (IC_{50} = 0.26 μM), T cell proliferation (54% inhibition at 10 μM), and mixed-lymphocyte reactions (IC_{50} = 3.1 μM).[177]

Cephalimysins A–D (**179–182**) along with FD-838 (**183**) were isolated from *Aspergillus fumigatus* OUPS-T106B-5 originally obtained from the marine fish *Mugil cephalus*.[183,184] Cephalimysins B–D are diastereomers of **183** which was originally isolated from the broth of an *Aspergillus* sp. in 1987 and its spirobicyclic core structure was constructed by enantioselective synthesis.[185] The initially assigned *trans* relationship between the C-8 methoxy group and C-9 alcohol structure of **179** was incorrect, but was revised by Yamada *et al.* in 2010 and subsequently confirmed by total synthesis in 2013.[184,186] Among the five compounds, **179** was found to display significant cytotoxic activity against P388 (IC_{50} = 15.0 nM) and HL-60 cell lines (IC_{50} = 9.5 nM);[183] **181–183** showed moderate activity toward both cell lines, although **180** was found to be inactive.[184] Only **183** was found to exert cytotoxicity (albeit slight) against L1210 leukemia, and human KB epidermoid carcinoma cell lines.[184]

Azaspirofurans A (**184**) and B (**185**) were obtained from the marine derived fungus *Aspergillus sydowi* D2-6.[187] Compound **184** exhibited moderate cytotoxic activity toward cancer cell line A549 with an IC_{50} value of 10 μM.[187]

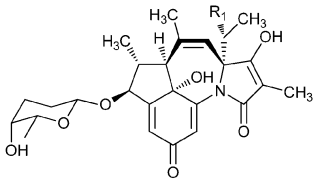
3.8 Other tetramic acid natural products

During the screening of soybean lipoxygenase inhibitor candidates, Komoda and co-workers discovered tetrapetalones A–D (**186–189**) from the culture filtrate of *Streptomyces* sp. USF-4727 isolated from a soil sample from Yada, Shizuoka City, Japan.[188,189] Initial structure elucidation of **186** was found to be incorrect and was later revised using ^{1}H–^{15}N HMBC techniques.[190] Tetrapetalone A (**186**) was found to contain a new tetracyclic skeleton and a β-D-rhodinosyl moiety, and its absolute stereochemistry was assigned on the basis of NOESY data and application of a modified Mosher's method.[188,190] The tetracyclic core of **186** has been synthesized by three groups.[191–193] Compounds **186–189** have been found to inhibit soybean lipoxygenase with IC_{50} values of 190, 320, 360 and 340 μM, respectively.[188,189] Quite interestingly, the C-3 methylated product was found to induce very little soybean lipoxygenase inhibition, even at 1 mM. This finding implies that the C-3 hydroxyl group likely plays a key role in lipoxygenase inhibition by this class of compounds.

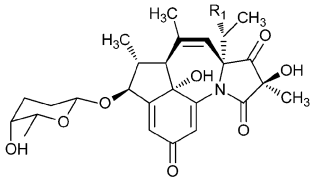
Pachydermin (**190**), an unusual oxalylated tetramic acid, was isolated from the methanol extract of the New Zealand basidiomycete *Chamonixia pachydermis* as a mixture of water-soluble Na^+ and K^+ salts.[194] Pachydermin was found to be very prone to degradation to 5-(3-chloro-4-hydroxybenzylidene)tetramic acid (**191**) under acidic conditions (0.05% TFA).[194] The bioactivity of **190** has not been assayed although the degradation product **191** appears to weakly inhibit *B. subtilis* in agar diffusion tests.[194]

Vermelhotin (**192**) was originally isolated as the *E*-isomer, along with antifungal compounds dihydroepiheveadride and deoxoepiheveadride, from the $CHCl_3$: MeOH (1 : 1) extract of an unidentified fungus IFM52672.[195] Subsequently, **192** was obtained as a mixture of *E* and *Z*-isomers from an unidentified fungus CRI247-01 and an endophytic fungus MEXU 26343 originating from the medical plant *Hintonia latiflora*.[196,197] The chemical structure of **192** was elucidated on the basis of spectroscopic data and X-ray crystallographic analyses. ^{1}H NMR data indicated that **192** spontaneously undergoes interconversion between *E* and *Z*-isomers, forming an equilibrium *E*/*Z* mixture (*E*/*Z* = 1 : 2 in DMSO-d_6, *E*/*Z* = 6.4 : 3.6 in $CDCl_3$).[196,197] Notably, this equilibrating *E*/*Z* mixture could not be separated by HPLC.[196,197] Compound **192** is an inhibitor of calmodulin and simulated docking studies have shown that **192** binds to calmodulin at site I. Compound **192** has been found to exert potent cytotoxicity against a panel of cancer cell lines with the IC_{50} in the range 0.31–13.5 μg mL^{-1}.[196,197] Natural product **190** also has shown antiplasmodial activity (IC_{50} = 1–10 μM), although it has failed to display antifungal and antibacterial activities.[195]

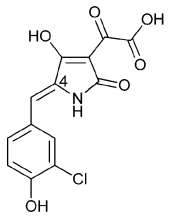
Che and co-workers obtained rigidiusculamides A–D (**193–196**) from the ascomycete fungus *Albonectria rigidiuscula* isolated from twigs of an identified tree on Jigong Mountain, China.[198] The 3*S*, 4*S*, 5*S* configuration of **193** determined using Snatzke's dimolybdenum CD method was found to be incorrect and was later revised to 3*S*, 4*S*, 5*R* as informed by its total synthesis; the absolute stereochemistries of **194–196** are 3*S*, 4*S*, 5*S*.[198,199] The opposite configurations of **193** and **194** are



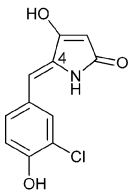
186 tetrapetalone A: R_1 = H
187 tetrapetalone B: R_1 = $OCOCH_3$



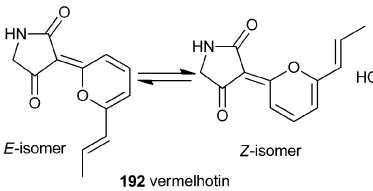
188 tetrapetalone C: R_1 = H
189 tetrapetalone D: R_1 = $OCOCH_3$



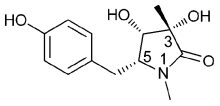
190 pachydermin



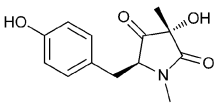
191



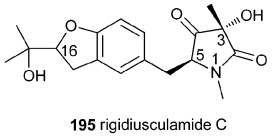
E-isomer *Z*-isomer
192 vermelhotin



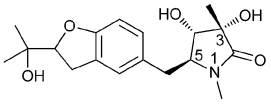
193 rigidiusculamide A



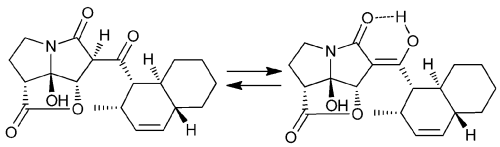
194 rigidiusculamide B



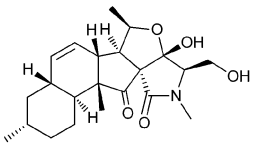
195 rigidiusculamide C



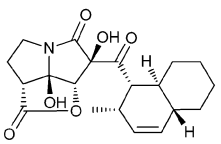
196 rigidiusculamide D



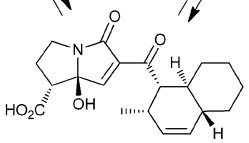
UCS1025 A(a) UCS1025 A(b)



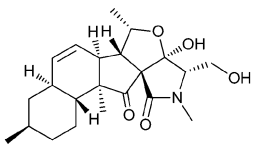
(-)-fusarisetin A



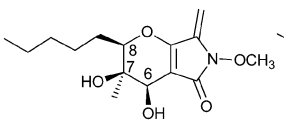
198 UCS1025B



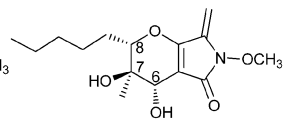
197 UCS1025 A(c)



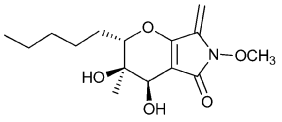
199 (+)-fusarisetin A



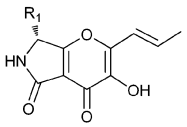
200a phaeosphaeride Aa



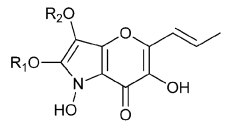
200b phaeosphaeride Ab



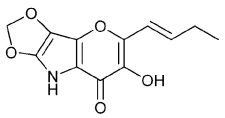
201 phaeosphaeride B



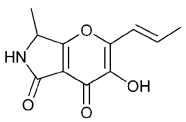
202 pyranonigrin A: R_1 = OH
206 pyranonigrin S: R_1 = H



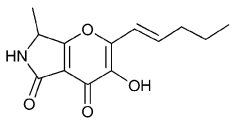
203 pyranonigrin B: R_1 = H, R_2 = CH_3
204 pyranonigrin C: R_1 = CH_3, R_2 = H



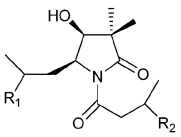
205 pyranonigrin D



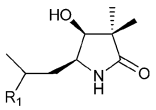
207 pyranonigrin E



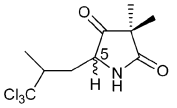
208 cordylactam



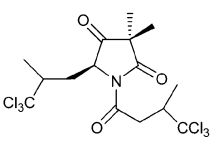
209 dysidamide D: R_1 = CCl_3, R_2 = $CHCl_2$
210 dysidamide E: R_1 = $CHCl_2$, R_2 = CCl_3
214 dysidamide: R_1 = CCl_3, R_2 = CCl_3
215 dysidamide B: R_1 = $CHCl_2$, R_2 = $CHCl_2$



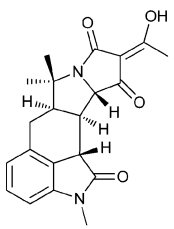
216 dysidamide C: R_1 = $CHCl_2$
211 dysidamide F: R_1 = CCl_3



212a dysidamide G: C_5 (*S*)
212b 5-*epi*-dysidamide G: C_5 (*R*)



213 dysidamide H



217 speradine A

consistent with an epimerization reaction that takes place after the generation of rigidiusculamide A. Compounds **193** and **194** have been found to exert moderate cytotoxicity against HeLa and MCF-7 cell lines with IC_{50} values ranging from 48.2–100.4 μg mL^{-1}, whereas, **195** and **196** are inactive against the same cell lines even up to concentrations of 120 μg mL^{-1}.[198] This discrepant activity seems to imply that the C-16 substituent dramatically influences cytotoxic activity.

UCS1025A (**197**) and B (**198**), harboring pentacyclic polyketides with a unique furopyrrolizidine skeleton, were isolated from the broth of fungus *Acremonium* sp. KY4917, and their structures and stereochemistries assigned on the basis of NMR and X-ray crystallographic analyses; assignments generated as a result of these analyses were ultimately confirmed by total synthesis.[200–202] Interesting, **197** exists as a mixture of three tautomeric isomers.[201] Compound **198** showed activity against *S. aureus*, *B. subtilis*, *E. hirae* and *Proteus vulgaris* with MICs in range of 1.3–5.2 μg mL^{-1}, and is much more potent than **198** (MICs = 42–83 μg mL^{-1}).[200] Compound **197** was also found to exert moderate antiproliferative activities against human tumor cell lines ACHN, A431, MCF-7 and T24 with IC_{50} values ranging from 21–58 μM.[200] Additionally, **197** (in contrast to **198**) was found to be a potent inhibitor of human telomerase displaying an IC_{50} of 1.3 μM.[203]

Fusarisetin A (**199**), possesses an unprecedented pentacyclic ring system, and was originally identified from a soil fungus *Fusarium* sp. FN080326 by application of a three-dimensional matrigel-induced acinar morphogenesis assay system in 2011.[204] The initially assigned absolute configuration of natural fusarisetin A was found to be incorrect,[204] although it was later revised as (+)-fusarisetin A on the basis of total synthesis data.[205–207] Compound **199** was found to inhibit 3D matrigel-induced acinar morphogenesis, cell migration, and serum-induced cell invasion. It was also found to reduce tube formation at concentrations of 30, 1, 10, and 30 μg mL^{-1}, respectively.[204] Additionally, **199** showed no significant cytotoxicity when evaluated with MDA-MB-231 cells at a concentration of 30 μg mL^{-1}.[204] Further investigations into proteomic profiling indicated that the molecular target of **199** is different from well-known signal pathway targets thus suggesting that **199** is a novel and promising anticancer agent representative of a potentially highly important scaffold.[204]

Phaeosphaeride A (**200**) and phaeosphaeride B (**201**) were isolated from the endophytic fungi *Phaeosphaeria avenaria* FA39 originated from plant samples collected in the Archbold biological station, Florida.[208] The originally reported structure of phaeosphaeride A (**200a**) has been found to be incorrect; NMR spectra of synthetic and naturally produced phaeosphaeride A are not identical. Hence, the structure of naturally isolated phaeosphaeride A is likely to be phaeosphaeride Ab (**200b**) or its enantiomer.[208–210] Bioactivity studies have shown that phaeosphaeride A is a selective signal transducer and activator of transcription factor 3 (STAT3) inhibitor. Moreover, the compound has shown potent activity against STAT3-dependent U266 multiple myeloma cells (IC_{50} = 6.7 μM). Notably however, **200** has also been found to be inactive against STAT3.[208]

Pyranonigrin A (**202**) along with B–D (**203–205**) were originally isolated from sponge-derived fungus *A. niger* Van Tieghem and was also obtained from extracts of marine fungus *A. niger* LL-LV3020.[211,212] Subsequently, **202** along with pyranonigrin S (**206**) was discovered from the methanol extracts of rice mold starters fermented by *A. saitoi*, *A. awamori*, *A. kawachii* and *A. oryzae* KCCM 60345.[213–215] Recently, pyranonigrin E (**207**), together with **206** and **202**, was isolated from *A. niger* NBRC5374 and *A. niger* ATCC 1015 respectively.[216] The original structure of pyranonigrin A was incorrect and was later revised by Schlingmann *et al.* in 2007.[212] Compound **202** showed no antimicrobial activity, no toxicity toward brine shrimp, and no antiproliferative activities against various leukemia and carcinoma cell lines. However, it has been found to exert significant inhibitory effects against the neonate larval growth of the plant insect *Spodoptera littoralis* and weak Epstein–Barr virus early antigen inhibitory activity.[211,217] Furthermore, 1,1-diphenyl-2-picryhydrozyl (DPPH) radical-scavenging assays revealed that pyranonigrin A, S and E are antioxidants, and that **206** exhibits a higher level of DPPH radical scavenging activity than either **202** or **207**.[213,214,216] In addition, **202** has been found to significantly suppress the expression of vascular cell adhesion molecule-1 in human umbilical vein endothelial cells (HUVECs) induced by tumor necrosis factor-α without affecting the cell viability of HUVECs.[214]

Cordylactam (**208**) was isolated from the culture of spider pathogenic fungus *Cordyceps* sp. BCC 12671, and its structure was determined by analyzing the multiple spectra and its semisynthetic derivative.[218] The biological activities of **208** have not yet been rigorously investigated although the crude extracts of *Cordyceps* sp. BCC 12671 have shown no bioactivity thus far.[218]

Five new natural polychlorinated pyrrolidinones dysidamide D–H (**209–213**) along with the previously reported dysidamide (**214**) and dysidamide B and C (**215**, **216**) were isolated from the dichloromethane extract of marine sponge *Lamellodysidea herbacea* collected from the Red Sea.[219] Dysidamide F (**211**) was first reported as a hydrolysis product of **214**.[220] ^1H NMR signals and X-ray crystal data indicate that dysidamide G exists as a pair of diastereoisomer mixtures in a 3 : 1 proportion.[219] From a biological perspective, dysidamide (**214**) has been found to show potent neurotoxic effects towards both mesencephalic and cortical murine neurons at a concentration of 0.8 μg mL^{-1}.[219]

Speradine A (**217**), a pentacyclic oxindole alkaloid, was obtained from the broth of *A. tamarii* isolated from driftwood at the seashore of Okinawa, Japan.[221] Its structural layout and relative stereochemistry were assigned on the basis of a series of spectroscopic and a single crystal X-ray diffraction data sets.[221] The compound's structural similarity to cyclopiazonic acid, explains, at least in part, why **217** is a potent inhibitor of Ca^{2+}-ATPase (IC_{50} = 8 μM). In addition, **217** has shown inhibitory activity against histone deacetylase (IC_{50} = 100 μg mL^{-1}) and antibacterial activity against *Mycrococcus Luteus* (MIC 16.7 μg mL^{-1}).[221]

4. Conclusions and outlooks

A comprehensive survey of known tetramic acid natural products arranged according to similarities in structural characteristics has been presented. These compounds are highly prominent representatives involved in and resulting from secondary metabolism in bacteria and fungi and are sometimes also found in sponges. Ongoing research has enabled the discovery of a rapidly increasing number and diversity of tetramic acid compounds. The tetramates contain intriguing structural features that often give rise to useful biological activities resulting from what are often highly novel mechanisms of action. Predicated on these grounds of structural complexity and enormous biomedical potential these natural products have attracted significant attention from the organic synthesis community. The urgency of new scaffold production and applications in the clinic has provided significant motivation for synthetic study of the tetramates both from the perspective of total synthesis but perhaps more importantly, from the perspective of producing analogs. Analog generation, in particular has proven useful from a standpoint of understanding mode of action and SAR data for any given compound class. Total synthesis, on the other hand, has very often been instrumental in achieving complete and accurate structural characterization of assorted tetramic acids natural products. Indeed, as highlighted here, an often undervalued virtue of total synthesis lies in its ability to help elucidate natural product structure and, by default quite often, mechanisms of action.

Although no tetramic acid-based compound or related congeners have yet been introduced into the clinic, it is worth noting that one synthetic derivative has been commercialized for the purposes of agricultural pest control.[222] In this case, the tetramate of interest is produced by its microbial producer and the biological activity of the tetramate may be associated with the need of the microbial producer to defend itself from neighboring detrimental microbes. Sometimes, the tetramic acid compounds can form a complex with metal irons such as Fe^{3+} or Zn^{2+} and this has sometimes been found to profoundly alter bioactivity. Indeed, our understanding of such concepts and the application of these ideas, is likely to evolve with continued advances in the area of the tetramic acid natural products. The past decade has provided many examples of the rich chemistry on display with naturally occurring tetramates and we anticipate that advances in this area will continue especially as advances in fields such as spectroscopy, synthetic methods, bioassays development, and biosynthetic mining and engineering continue also to evolve at their presently rapid paces. Just as the last decade has seen dramatic advances in our understanding of the tetramic acids we expect that the next decade will provide even more dramatic advances.

Acknowledgements

Research on in our laboratory was supported by grants from the National Natural Science Foundation of China (31300063), Natural Science Foundation of Shandong Province (ZR2013CL020), National High Technology Research and Development Program of China (2012AA092104), National Basic Research Program of China (2010CB833805), and CAS (XDA11030403).

References

1 B. J. Royles, *Chem. Rev.*, 1995, **95**, 1981–2001.
2 E. L. Ghisalberti, *Studies in natural products chemistry*, 2003, vol. 28, pp. 109–163.
3 A. Gossauer, in *Fortschritte der Chemie organischer Naturstoffe/Progress in the Chemistry of Organic Natural Products*, Springer, 2003, pp. 1–188.
4 R. Schobert and A. Schlenk, *Bioorg. Med. Chem.*, 2008, **16**, 4203–4221.
5 D. Boettger and C. Hertweck, *ChemBioChem*, 2013, **14**, 28–42.
6 K. M. Fisch, *RSC Adv.*, 2013, **3**, 18228–18247.
7 M. Kawada, Y. Yoshimoto, H. Kumagai, T. Someno, I. Momose, N. Kawamura, K. Isshiki and D. Ikeda, *J. Antibiot.*, 2004, **57**, 235–237.
8 T. Rosett, R. Sankhala, C. Stickings, M. Taylor and R. Thomas, *Biochem. J.*, 1957, **67**, 390–400.
9 S. Gatenbeck and J. Sierankiewicz, *Antimicrob. Agents Chemother.*, 1973, **3**, 308–309.
10 F. A. Miller, W. A. Rightsel, B. J. Sloan, J. Ehrlich, J. C. French, Q. R. Bartz and G. J. Dixon, *Nature*, 1963, **200**, 1338–1339.
11 N. Davis, U. Diener and G. Morgan-Jones, *Appl. Environ. Microbiol.*, 1977, **34**, 155–157.
12 M. Nukina and T. Saito, *Biosci., Biotechnol., Biochem.*, 1992, **56**, 1314–1315.
13 B. Schlegel, M. Schmidtke, H. Dörfelt, P. Kleinwächter and U. Gräfe, *J. Basic Microbiol.*, 2001, **41**, 179–183.
14 V. Hellwig, T. Grothe, A. Mayer-Bartschmid, R. Endermann, F.-U. Geschke, T. Henkel and M. Stadler, *J. Antibiot.*, 2002, **55**, 881–892.
15 G. L. Gallardo, N. I. Peña, P. Chacana, H. R. Terzolo and G. M. Cabrera, *World J. Microbiol. Biotechnol.*, 2004, **20**, 609–612.
16 E. C. Marfori, S. i. Kajiyama, E.-i. Fukusaki and A. Kobayashi, *Phytochemistry*, 2003, **62**, 715–721.
17 K. Tylkowska, J. Grabarkiewicz-Szczesna and H. Iwanowska, *Seed Sci. Technol.*, 2003, **31**, 309–316.
18 S. Chen, X. Xu, X. Dai, C. Yang and S. Qiang, *Biochim. Biophys. Acta*, 2007, **1767**, 306–318.
19 S. Chen, F. Zhou, C. Yin, R. J. Strasser, C. Yang and S. Qiang, *Environ. Exp. Bot.*, 2011, **71**, 269–279.
20 S. Chen, C. Yin, R. J. Strasser, C. Yang and S. Qiang, *Plant Physiol. Biochem.*, 2012, **52**, 38–51.
21 K. Fukunaga, T. Misato, I. Ishii and M. Asakawa, *Bull. Agric. Chem. Soc. Jpn.*, 1955, **19**, 181–188.
22 S. Sakuda, H. Ikeda, T. Nakamura, R. Kawachi, T. Kondo, M. Ono, M. Sakurada, H. Inagaki, R. Ito and H. Nagasawa, *J. Antibiot.*, 2000, **53**, 1378–1384.
23 S. Sakuda, H. Ikeda, T. Nakamura and H. Nagasawa, *Biosci., Biotechnol., Biochem.*, 2004, **68**, 407–412.

24 S. Sakuda, N. Matsumori, K. Furihata and H. Nagasawa, *Tetrahedron Lett.*, 2007, **48**, 2527–2531.
25 S. Sakuda, M. Ono, K. Furihata, J. Nakayama, A. Suzuki and A. Isogai, *J. Am. Chem. Soc.*, 1996, **118**, 7855–7856.
26 M. Ono, S. Sakuda, A. Suzuki and A. Isogai, *J. Antibiot.*, 1997, **50**, 111–118.
27 T. Yoshinari, Y. Noda, K. Yoda, H. Sezaki, H. Nagasawa and S. Sakuda, *J. Antibiot.*, 2010, **63**, 309–314.
28 A. D. Wright, C. Osterhage and G. M. König, *Org. Biomol. Chem.*, 2003, **1**, 507–510.
29 H. V. K. Wangun, H.-M. Dahse and C. Hertweck, *J. Nat. Prod.*, 2007, **70**, 1800–1803.
30 S. Loscher and R. Schobert, *Chem.–Eur. J.*, 2013, **19**, 10619–10624.
31 H. V. K. Wangun and C. Hertweck, *Org. Biomol. Chem.*, 2007, **5**, 1702–1705.
32 Y. Ujihara, K. Nakayama, T. Sengoku, M. Takahashi and H. Yoda, *Org. Lett.*, 2012, **14**, 5142–5145.
33 S. Aoki, K. Higuchi, Y. Ye, R. Satari and M. Kobayashi, *Tetrahedron*, 2000, **56**, 1833–1836.
34 C. Y. Wang, B. G. Wang, S. Wiryowidagdo, V. Wray, R. van Soest, K. G. Steube, H. S. Guan, P. Proksch and R. Ebel, *J. Nat. Prod.*, 2003, **66**, 51–56.
35 J. Xu, M. Hasegawa, K. Harada, H. Kobayashi, H. Nagai and M. Namikoshi, *Chem. Pharm. Bull.*, 2006, **54**, 852–854.
36 B. Biersack, R. Diestel, C. Jagusch, G. Rapp, F. Sasse and R. Schobert, *Chem. Biodiversity*, 2008, **5**, 2423–2430.
37 T. Oda, A. Fujita, J. Xu, M. Mochizuki, K. Ukai and M. Namikoshi, *Mar. Drugs*, 2007, **5**, 1–5.
38 R. Schobert, F. Sasse, B. Biersack, K. Effenberger, S. Breyer and R. Diestel, *Int. J. Clin. Pharmacol. Ther.*, 2010, **48**, 459–461.
39 B. Biersack, R. Diestel, C. Jagusch, F. Sasse and R. Schobert, *J. Inorg. Biochem.*, 2009, **103**, 72–76.
40 Z. J. Lin, Z. Y. Lu, T. J. Zhu, Y. C. Fang, Q. Q. Gu and W. M. Zhu, *Chem. Pharm. Bull.*, 2008, **56**, 217–221.
41 T. Sengoku, J. Wierzejska, M. Takahashi and H. Yoda, *Synlett*, 2010, 2944–2946.
42 T. Sengoku, Y. Nagae, Y. Ujihara, M. Takahashi and H. Yoda, *J. Org. Chem.*, 2012, **77**, 4391–4401.
43 K. Kempf, A. Raja, F. Sasse and R. Schobert, *J. Org. Chem.*, 2013, **78**, 2455–2461.
44 A. Höltzel, M. G. Gäenzle, G. J. Nicholson, W. P. Hammes and G. Jung, *Angew. Chem., Int. Ed.*, 2000, **39**, 2766–2768.
45 M. G. Gänzle, A. Höltzel, J. Walter, G. Jung and W. P. Hammes, *Appl. Environ. Microbiol.*, 2000, **66**, 4325–4333.
46 U. Marquardt, D. Schmid and G. Jung, *Synlett*, 2000, 1131–1132.
47 R. Böhme, G. Jung and E. Breitmaier, *Helv. Chim. Acta*, 2005, **88**, 2837–2841.
48 M. G. Gänzle and R. F. Vogel, *Appl. Environ. Microbiol.*, 2003, **69**, 1305–1307.
49 M. Gänzle, *Appl. Microbiol. Biotechnol.*, 2004, **64**, 326–332.
50 J. G. Hurdle, A. E. Heathcott, L. Yang, B. Yan and R. E. Lee, *J. Antimicrob. Chemother.*, 2011, **66**, 1773–1776.
51 J. G. Hurdle, R. Yendapally, D. Sun and R. E. Lee, *Antimicrob. Agents Chemother.*, 2009, **53**, 4028–4031.
52 G. F. Kaufmann, R. Sartorio, S. H. Lee, C. J. Rogers, M. M. Meijler, J. A. Moss, B. Clapham, A. P. Brogan, T. J. Dickerson and K. D. Janda, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 309–314.
53 C. Ueda, K. Tateda, M. Horikawa, S. Kimura, Y. Ishii, K. Nomura, K. Yamada, T. Suematsu, Y. Inoue, M. Ishiguro, S. Miyairi and K. Yamaguchi, *Antimicrob. Agents Chemother.*, 2010, **54**, 683–688.
54 N. H. Honda, S. Kimura, K. Tateda, M. Horikawa, C. Ueda, Y. Ishii, M. Ishiguro, S. Miyairi and K. Yamaguchi, *Microbes Environ.*, 2011, **26**, 160–164.
55 C. A. Lowery, J. Park, C. Gloeckner, M. M. Meijler, R. S. Mueller, H. I. Boshoff, R. L. Ulrich, C. E. Barry III, D. H. Bartlett, V. V. Kravchenko, G. F. Kaufmann and K. D. Janda, *J. Am. Chem. Soc.*, 2009, **131**, 14473–14479.
56 A. A. Romano, T. Hahn, N. Davis, C. A. Lowery, A. K. Struss, K. D. Janda, L. H. Böttger, B. F. Matzanke and C. J. Carrano, *J. Inorg. Biochem.*, 2012, **107**, 96–103.
57 W. Steglich, *Pure Appl. Chem.*, 1989, **61**, 281–288.
58 N. Riache, C. Bailly, A. Deville, L. Dubost and B. Nay, *Eur. J. Org. Chem.*, 2010, 5402–5408.
59 C. Hopmann, M. Kurz, M. Brönstrup, J. Wink and D. LeBeller, *Tetrahedron Lett.*, 2002, **43**, 435–438.
60 J. Ondeyka, G. Harris, D. Zink, A. Basilio, F. Vicente, G. Bills, G. Platas, J. Collado, A. González, M. de la Cruz, J. Martin, J. N. Kahn, S. Galuska, R. Giacobbe, G. Abruzzo, E. Hickey, P. Liberator, B. Jiang, D. Xu, T. Roemer and S. B. Singh, *J. Nat. Prod.*, 2008, **72**, 136–141.
61 M. Figueroa, H. Raja, J. O. Falkinham III, A. F. Adcock, D. J. Kroll, M. C. Wani, C. J. Pearce and N. H. Oberlies, *J. Nat. Prod.*, 2013, **76**, 1007–1015.
62 A. Yajima, C. Ida, K. Taniguchi, S. Murata, R. Katsuta and T. Nukada, *Tetrahedron Lett.*, 2013, **54**, 2497–2501.
63 M. Wheeler, R. Stipanovic and L. Puckhaber, *Mycol. Res.*, 1999, **103**, 967–973.
64 S. P. Putri, H. Kinoshita, F. Ihara, Y. Igarashi and T. Nihira, *J. Antibiot.*, 2010, **63**, 195–198.
65 Y. Sugie, S. Inagaki, Y. Kato, H. Nishida, C.-H. Pang, T. Saito, S. Sakemi, F. Dib-Hajj, J. P. Mueller, J. Sutcliffe and Y. Kojima, *J. Antibiot.*, 2002, **55**, 25–29.
66 E. C. Marfori, S. Kajiyama, E. Fukusaki and A. Kobayashi, *Z. Naturforsch., C: Biosci.*, 2002, **57**, 465–470.
67 J. Inokoshi, N. Shigeta, T. Fukuda, R. Uchida, K. Nonaka, R. Masuma and H. Tomoda, *J. Antibiot.*, 2013, **66**, 549–554.
68 E. C. Marfori and A. B. Malasa, *Philippine Agr. Sci.*, 2007, **90**, 91–95.
69 P. A. O. Alad, A. A. S. Cocos, E. C. Balolong Jr, M. M. Parungao and E. C. Marfori, *Philippine J. Sci.*, 2009, **138**, 119–124.
70 Y. Sugie, K. A. Dekker, T. Inagaki, Y.-J. Kim, T. Sakakibara, S. Sakemi, A. Sugiura, L. Brennan, J. Duignan, J. A. Sutcliffe and Y. Kojima, *J. Antibiot.*, 2002, **55**, 19–24.
71 M. Daferner, T. Anke and O. Sterner, *Tetrahedron*, 2002, **58**, 7781–7784.

72 G. Lang, J. W. Blunt, N. J. Cummings, A. L. Cole and M. H. Munro, *J. Nat. Prod.*, 2005, **68**, 810–811.
73 K. Herath, H. Jayasuriya, D. L. Zink, J. Sigmund, F. Vicente, M. de la Cruz, A. Basilio, G. F. Bills, J. D. Polishook, R. Donald, J. Phillips, M. Goetz and S. B. Singh, *J. Nat. Prod.*, 2012, **75**, 420–424.
74 S.-W. Yang, R. Mierzwa, J. Terracciano, M. Patel, V. Gullo, N. Wagner, B. Baroudy, M. Puar, T.-M. Chan, A. T. McPhail and M. Chu, *J. Nat. Prod.*, 2006, **69**, 1025–1028.
75 S.-W. Yang, R. Mierzwa, J. Terracciano, M. Patel, V. Gullo, N. Wagner, B. Baroudy, M. Puar, T.-M. Chan and M. Chu, *J. Antibiot.*, 2007, **60**, 524–528.
76 K. Neumann, S. Kehraus, M. Gütschow and G. König, *Nat. Prod. Commun.*, 2009, **4**, 347–354.
77 T. Amagata, J. Xiao, Y.-P. Chen, N. Holsopple, A. G. Oliver, T. Gokey, A. B. Guliaev and K. Minoura, *J. Nat. Prod.*, 2012, **75**, 2193–2199.
78 M. Izumikawa, J. Hashimoto, T. Hirokawa, S. Sugimoto, T. Kato, M. Takagi and K. Shin-ya, *J. Nat. Prod.*, 2010, **73**, 628–631.
79 T. Watanabe, M. Igarashi, T. Okajima, E. Ishii, H. Kino, M. Hatano, R. Sawa, M. Umekita, T. Kimura, S. Okamoto, Y. Eguchi, Y. Akamatsu and R. Utsumi, *Antimicrob. Agents Chemother.*, 2012, **56**, 3657–3663.
80 N. C. H. Quek, J. H. Matthews, S. Bloor, D. Jones, P. W. Bircham, R. Heathcott and P. H. Atkinson, *Mol. BioSyst.*, 2013, **9**, 2125–2133.
81 S. B. Singh, D. L. Zink, B. Heimbach, O. Genilloud, A. Teran, K. C. Silverman, R. B. Lingham, P. Felock and D. J. Hazuda, *Org. Lett.*, 2002, **4**, 1123–1126.
82 J. W. Phillips, M. A. Goetz, S. K. Smith, D. L. Zink, J. Polishook, R. Onishi, S. Salowe, J. Wiltsie, J. Allocco, J. Sigmund, K. Dorso, S. Lee, S. Skwish, M. de la Cruz, J. Martín, F. Vicente, O. Genilloud, J. Lu, R. E. Painter, K. Young, K. Overbye, R. G. Donald and S. B. Singh, *Chem. Biol.*, 2011, **18**, 955–965.
83 S. B. Singh, M. A. Goetz, S. K. Smith, D. L. Zink, J. Polishook, R. Onishi, S. Salowe, J. Wiltsie, J. Allocco, J. Sigmund, K. Dorso, M. de la Cruz, J. Martín, F. Vicente, O. Genilloud, R. G. Donald and J. W. Phillips, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 7127–7130.
84 R. Sawa, Y. Takahashi, H. Hashizume, K. Sasaki, Y. Ishizaki, M. Umekita, M. Hatano, H. Abe, T. Watanabe, N. Kinoshita, Y. Homma, C. Hayashi, K. Inoue, S. Ohba, T. Masuda, M. Arakawa, Y. Kobayashi, M. Hamada, M. Igarashi, H. Adachi, Y. Nishimura and Y. Akamatsu, *Chem.–Eur. J.*, 2012, **18**, 15772–15781.
85 S. Tohyama, Y. Takahashi and Y. Akamatsu, *J. Antibiot.*, 2010, **63**, 147–149.
86 T. Furumai, K. Eto, T. Sasaki, H. Higuchi, H. Onaka, N. Saito, T. Fujita, H. Naoki and Y. Igarashi, *J. Antibiot.*, 2002, **55**, 873–880.
87 R. Sawa, Y. Mori, H. Iinuma, H. Naganawa, M. Hamada, S. Yoshida, H. Furutani, Y. Kajimura, T. Fuwa and T. Takeuchi, *J. Antibiot.*, 1994, **47**, 731–732.
88 F. Vinale, G. Flematti, K. Sivasithamparam, M. Lorito, R. Marra, B. W. Skelton and E. L. Ghisalberti, *J. Nat. Prod.*, 2009, **72**, 2032–2035.
89 F. Vinale, M. Nigro, K. Sivasithamparam, G. Flematti, E. L. Ghisalberti, M. Ruocco, R. Varlese, R. Marra, S. Lanzuise, A. Eid, S. L. Woo and M. Lorito, *FEMS Microbiol. Lett.*, 2013, **347**, 123–129.
90 C. Deboer, A. Dietz, G. Savage and W. Silver, *Antibiot. Annu.*, 1955, **3**, 886–892.
91 C. Olano, C. Gómez, M. Pérez, M. Palomino, A. Pineda-Lucena, R. J. Carbajo, A. F. Braña, C. Méndez and J. A. Salas, *Chem. Biol.*, 2009, **16**, 1031–1044.
92 D. H. Horna, C. Gómez, C. Olano, M. Palomino-Schätzlein, A. Pineda-Lucena, R. J. Carbajo, A. F. Braña, C. Méndez and J. A. Salas, *J. Bacteriol.*, 2011, **193**, 2647–2651.
93 C. Gómez, D. H. Horna, C. Olano, M. Palomino-Schätzlein, A. Pineda-Lucena, R. J. Carbajo, A. F. Braña, C. Méndez and J. A. Salas, *J. Bacteriol.*, 2011, **193**, 4214–4223.
94 S. V. Pronin and S. A. Kozmin, *J. Am. Chem. Soc.*, 2010, **132**, 14394–14396.
95 S. V. Pronin, A. Martinez, K. Kuznedelov, K. Severinov, H. A. Shuman and S. A. Kozmin, *J. Am. Chem. Soc.*, 2011, **133**, 12172–12184.
96 D. Temiakov, N. Zenkin, M. N. Vassylyeva, A. Perederina, T. H. Tahirov, E. Kashkina, M. Savkina, S. Zorov, V. Nikiforov, N. Igarashi, N. Matsugaki, S. Wakatsuki, K. Severinov and D. G. Vassylyev, *Mol. Cell*, 2005, **19**, 655–666.
97 S. Tuske, S. G. Sarafianos, X. Wang, B. Hudson, E. Sineva, J. Mukhopadhyay, J. J. Birktoft, O. Leroy, S. Ismail, A. D. Clark Jr, C. Dharia, A. Napoli, O. Laptenko, J. Lee, S. Borukhov, R. H. Ebright and E. Arnold, *Cell*, 2005, **122**, 541–552.
98 C. E. Meyer, *J. Antibiot.*, 1971, **24**, 558–560.
99 H. Hagenmaier, K. Jaschke, L. Santo, M. Scheer and H. Zähner, *Arch. Microbiol.*, 1976, **109**, 65–74.
100 J. C. Carlson, S. Li, D. A. Burr and D. H. Sherman, *J. Nat. Prod.*, 2009, **72**, 2076–2079.
101 C. Duan, Y. Yao, Z. Wang, X. Tian, S. Zhang, C. Zhang and J. Ju, *Chin. J. Mar. Drugs*, 2010, **29**, 12–20.
102 Z. Yu, S. Vodanovic-Jankovic, N. Ledeboer, S.-X. Huang, S. R. Rajski, M. Kron and B. Shen, *Org. Lett.*, 2011, **13**, 2034–2037.
103 M. E. Rateb, Z. Yu, Y. Yan, D. Yang, T. Huang, S. Vodanovic-Jankovic, M. A. Kron and B. Shen, *J. Antibiot.*, 2014, **67**, 127–132.
104 A. Espinosa, A. M. Socha, E. Ryke and D. C. Rowley, *Parasitol. Res.*, 2012, **111**, 2473–2477.
105 X. Mo, Z. Wang, B. Wang, J. Ma, H. Huang, X. Tian, S. Zhang, C. Zhang and J. Ju, *Biochem. Biophys. Res. Commun.*, 2011, **406**, 341–347.
106 X. Mo, H. Huang, J. Ma, Z. Wang, B. Wang, S. Zhang, C. Zhang and J. Ju, *Org. Lett.*, 2011, **13**, 2212–2215.
107 J. C. Carlson, J. Fortman, Y. Anzai, S. Li, D. A. Burr and D. H. Sherman, *ChemBioChem*, 2010, **11**, 564–572.

108 J. C. Carlson, S. Li, S. S. Gunatilleke, Y. Anzai, D. A. Burr, L. M. Podust and D. H. Sherman, *Nat. Chem.*, 2011, **3**, 628–633.
109 X. Mo, J. Ma, H. Huang, B. Wang, Y. Song, S. Zhang, C. Zhang and J. Ju, *J. Am. Chem. Soc.*, 2012, **134**, 2844–2847.
110 D. J. Duchamp, A. R. Branfman, A. C. Button and K. L. Rinehart Jr, *J. Am. Chem. Soc.*, 1973, **95**, 4077–4078.
111 P. DeShong, S. Ramesh, V. Elango and J. J. Perez, *J. Am. Chem. Soc.*, 1985, **107**, 5219–5224.
112 S. J. Shimshock, R. E. Waltermire and P. DeShong, *J. Am. Chem. Soc.*, 1991, **113**, 8791–8796.
113 J. S. Yadav, S. Dhara, S. S. Hossain and D. K. Mohapatra, *J. Org. Chem.*, 2012, **77**, 9628–9633.
114 M. Chen and W. R. Roush, *Org. Lett.*, 2012, **14**, 426–428.
115 F. Reusser, *Antimicrob. Agents Chemother.*, 1976, **10**, 618–622.
116 S. Matsunaga, N. Fusetani, Y. Kato and H. Hirota, *J. Am. Chem. Soc.*, 1991, **113**, 9690–9692.
117 N. U. Sata, S. Matsunaga, N. Fusetani and R. W. van Soest, *J. Nat. Prod.*, 1999, **62**, 969–971.
118 D. Wolf, F. J. Schmitz, F. Qiu and M. Kelly-Borges, *J. Nat. Prod.*, 1999, **62**, 170–172.
119 A. S. Ratnayake, R. A. Davis, M. K. Harper, C. A. Veltri, C. D. Andjelic, L. R. Barrows and C. M. Ireland, *J. Nat. Prod.*, 2005, **68**, 104–107.
120 R. F. Angawi, G. Bavestrello, B. Calcinai, H. A. Dien, G. Donnarumma, M. A. Tufano, I. Paoletti, E. Grimaldi, G. Chianese, E. Fattorusso and O. Taglialatela-Scafati, *Mar. Drugs*, 2011, **9**, 2809–2817.
121 R. Kumar, R. Subramani, K.-D. Feussner and W. Aalbersberg, *Mar. Drugs*, 2012, **10**, 200–208.
122 K. Schmidt, U. Riese, Z. Li and M. Hamburger, *J. Nat. Prod.*, 2003, **66**, 378–383.
123 K. Schmidt, W. Günther, S. Stoyanova, B. Schubert, Z. Li and M. Hamburger, *Org. Lett.*, 2002, **4**, 197–199.
124 M. Isaka, P. Chinthanom, S. Supothina, P. Tobwor and N. L. Hywel-Jones, *J. Nat. Prod.*, 2010, **73**, 2057–2060.
125 A. P. Michael, E. J. Grace, M. Kotiw and R. A. Barrow, *J. Nat. Prod.*, 2002, **65**, 1360–1362.
126 A. Schlenk, R. Diestel, F. Sasse and R. Schobert, *Chem.–Eur. J.*, 2010, **16**, 2599–2604.
127 A. Nowak and B. Steffan, *Angew. Chem., Int. Ed.*, 1998, **37**, 3139–3141.
128 F. Blumenthal, K. Polborn and B. Steffan, *Tetrahedron*, 2002, **58**, 8433–8437.
129 D. A. Longbottom, A. J. Morrison, D. J. Dixon and S. V. Ley, *Angew. Chem., Int. Ed.*, 2002, **41**, 2786–2790.
130 D. A. Longbottom, A. J. Morrison, D. J. Dixon and S. V. Ley, *Tetrahedron*, 2003, **59**, 6955–6966.
131 T. Sugawara, H. Shinonaga, H. Simura, R. Yoshikawa and K. Yamamoto, *Jpn. Kokai Tokkyo Koho*, 1996, 319289.
132 H. Kakeya, S. Kageyama, L. Nie, R. Onose, G. Okada, T. Beppu, C. J. Norbury and H. Osada, *J. Antibiot.*, 2001, **54**, 850–854.
133 S. B. Krasnoff, C. H. Sommers, Y.-S. Moon, B. G. G. Donzelli, J. D. Vandenberg, A. C. L. Churchill and D. M. Gibson, *J. Agric. Food Chem.*, 2006, **54**, 7083–7088.
134 Y. Hayashi, J. Yamaguchi and M. Shoji, *Tetrahedron*, 2002, **58**, 9839–9846.
135 S. Kanazawa, N. Fusetani and S. Matsunaga, *Tetrahedron Lett.*, 1993, **34**, 1065–1068.
136 N. Cramer, S. Laschat, A. Baro, H. Schwalbe and C. Richter, *Angew. Chem., Int. Ed.*, 2005, **44**, 820–822.
137 N. Cramer, M. Buchweitz, S. Laschat, W. Frey, A. Baro, D. Mathieu, C. Richter and H. Schwalbe, *Chem.–Eur. J.*, 2006, **12**, 2488–2503.
138 A. C. Hart and A. J. Phillips, *J. Am. Chem. Soc.*, 2006, **128**, 1094–1095.
139 N. Cramer, S. Helbig, A. Baro, S. Laschat, R. Diestel, F. Sasse, D. Mathieu, C. Richter, G. Kummerlöwe, B. Luy and H. Schwalbe, *ChemBioChem*, 2008, **9**, 2474–2486.
140 P. R. Graupner, A. Carr, E. Clancy, J. Gilbert, K. L. Bailey, J.-A. Derby and B. C. Gerwick, *J. Nat. Prod.*, 2003, **66**, 1558–1561.
141 T. Yoshinari, K. Ohmori, M. G. Schrems, A. Pfaltz and K. Suzuki, *Angew. Chem.*, 2010, **122**, 893–897.
142 B. Barnickel and R. Schobert, *J. Org. Chem.*, 2010, **75**, 6716–6719.
143 L. J. Giesler and G. Y. Yuen, *Crop Prot.*, 1998, **17**, 509–513.
144 F. Yu, K. Zaleta-Rivera, X. Zhu, J. Huffman, J. C. Millet, S. D. Harris, G. Yuen, X.-C. Li and L. Du, *Antimicrob. Agents Chemother.*, 2007, **51**, 64–72.
145 Z. Zhang and G. Yuen, *Phytopathology*, 1999, **89**, 817–822.
146 G. Yuen, J. Steadman, D. Lindgren, D. Schaff and C. Jochum, *Crop Prot.*, 2001, **20**, 395–402.
147 D. Y. Kobayashi, R. M. Reedy, J. D. Palumbo, J.-M. Zhou and G. Y. Yuen, *Appl. Environ. Microbiol.*, 2005, **71**, 261–269.
148 D. Y. Kobayashi and G. Y. Yuen, *Can. J. Microbiol.*, 2005, **51**, 719–723.
149 Y. Li, J. Huffman, Y. Li, L. Du and Y. Shen, *Med. Chem. Commun.*, 2012, **3**, 982–986.
150 S. Li, C. Jochum, F. Yu, K. Zaleta-Rivera, L. Du, S. Harris and G. Yuen, *Phytopathology*, 2008, **98**, 695–701.
151 S. Li, L. Du, G. Yuen and S. D. Harris, *Mol. Biol. Cell*, 2006, **17**, 1218–1227.
152 J. A. Blodgett, D.-C. Oh, S. Cao, C. R. Currie, R. Kolter and J. Clardy, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 11692–11697.
153 S. Cao, J. A. Blodgett and J. Clardy, *Org. Lett.*, 2010, **12**, 4652–4654.
154 T. L. Simmons, K. L. McPhail, E. Ortega-Barría, S. L. Mooberry and W. H. Gerwick, *Tetrahedron Lett.*, 2006, **47**, 3387–3390.
155 H. Q. Lan, J. L. Ye, A. E. Wang, Y. P. Ruan and P. Q. Huang, *Chem.–Eur. J.*, 2011, **17**, 958–968.
156 G. Höfle, K. Gerth, H. Reichenbach, B. Kunze, F. Sasse, E. Forche and E. V. Prusov, *Chem.–Eur. J.*, 2012, **18**, 11362–11370.
157 Y. Schmidt and B. Breit, *Org. Lett.*, 2010, **12**, 2218–2221.
158 R. G. Linington, B. R. Clark, E. E. Trimble, A. Almanza, L.-D. Ureña, D. E. Kyle and W. H. Gerwick, *J. Nat. Prod.*, 2009, **72**, 14–17.
159 T. Conroy, J. T. Guo, R. G. Linington, N. H. Hunt and R. J. Payne, *Chem.–Eur. J.*, 2011, **17**, 13544–13552.

160 K. Taori, Y. Liu, V. J. Paul and H. Luesch, *ChemBioChem*, 2009, **10**, 1634–1639.
161 T. Conroy, J. T. Guo, N. H. Hunt and R. J. Payne, *Org. Lett.*, 2010, **12**, 5576–5579.
162 S. C. Stolze, E. Deu, F. Kaschani, N. Li, B. I. Florea, K. H. Richau, T. Colby, R. A. van der Hoorn, H. S. Overkleeft, M. Bogyo and M. Kaiser, *Chem. Biol.*, 2012, **19**, 1546–1555.
163 S. Suntornchashwej, K. Suwanborirux, K. Koga and M. Isobe, *Chem.–Asian J.*, 2007, **2**, 114–122.
164 S. Suntornchashwej, K. Suwanborirux and M. Isobe, *Tetrahedron*, 2007, **63**, 3217–3226.
165 K. E. Milligan, B. Márquez, R. T. Williamson, M. Davies-Coleman and W. H. Gerwick, *J. Nat. Prod.*, 2000, **63**, 965–968.
166 J. Chen, X.-G. Fu, L. Zhou, J.-T. Zhang, X.-L. Qi and X.-P. Cao, *J. Org. Chem.*, 2009, **74**, 4149–4157.
167 P. Phuwapraisirisan, S. Matsunaga and N. Fusetani, *Org. Lett.*, 2005, **7**, 2233–2236.
168 H. Luesch, W. Y. Yoshida, R. E. Moore and V. J. Paul, *Tetrahedron*, 2002, **58**, 7959–7966.
169 H.-Q. Lan, Y.-P. Ruan and P.-Q. Huang, *Chem. Commun.*, 2010, **46**, 5319–5321.
170 W.-J. Bai, S. K. Jackson and T. R. Pettus, *Org. Lett.*, 2012, **14**, 3862–3865.
171 M. D. Sadar, D. E. Williams, N. R. Mawji, B. O. Patrick, T. Wikanta, E. Chasanah, H. E. Irianto, R. V. Soest and R. J. Andersen, *Org. Lett.*, 2008, **10**, 4947–4950.
172 P. Bloch, C. Tamm, P. Bollinger, T. J. Petcher and H. P. Weber, *Helv. Chim. Acta*, 1976, **59**, 133–137.
173 J. Wenke, H. Anke and O. Sterner, *Biosci., Biotechnol., Biochem.*, 1993, **57**, 961–964.
174 Y. Igarashi, Y. Yabuta, A. Sekine, K. Fujii, K.-I. Harada, T. Oikawa, M. Sato, T. Furumai and T. Oki, *J. Antibiot.*, 2004, **57**, 748–754.
175 F.-Z. Wang, D.-H. Li, T.-J. Zhu, M. Zhang and Q.-Q. Gu, *Can. J. Chem.*, 2011, **89**, 72–76.
176 H. P. Weber, T. J. Petcher, P. Bloch and C. Tamm, *Helv. Chim. Acta*, 1976, **59**, 137–140.
177 M. Ishikawa, T. Ninomiya, H. Akabane, N. Kushida, G. Tsujiuchi, M. Ohyama, S. Gomi, K. Shito and T. Murata, *Bioorg. Med. Chem. Lett.*, 2009, **19**, 1457–1460.
178 Y. Asami, H. Kakeya, R. Onose, A. Yoshida, H. Matsuzaki and H. Osada, *Org. Lett.*, 2002, **4**, 2845–2848.
179 S. Aoki, T. Oi, K. Shimizu, R. Shiraki, K. Takao and K. Tadano, *Heterocycles*, 2004, **62**, 161–166.
180 Y. Asami, H. Kakeya, Y. Komi, S. Kojima, K. Nishikawa, K. Beebe, L. Neckers and H. Osada, *Cancer Sci.*, 2008, **99**, 1853–1858.
181 O. Ando, H. Satake, M. Nakajima, A. Sato, T. Nakamura, T. Kinoshita, K. Furuya and T. Haneishi, *J. Antibiot.*, 1991, **44**, 382–389.
182 (*a*) Y. Igarashi, Y. Yabuta and T. Furumai, *J. Antibiot.*, 2004, **57**, 537–540; (*b*) Y. Hayashi, M. Shoji, T. Mukaiyama, H. Gotoh, S. Yamaguchi, M. Nakata, H. Kakeya and H. Osada, *J. Org. Chem.*, 2005, **70**, 5643–5654.
183 T. Yamada, E. Imai, K. Nakatuji, A. Numata and R. Tanaka, *Tetrahedron Lett.*, 2007, **48**, 6294–6296.
184 T. Yamada, H. Kitada, T. Kajimoto, A. Numata and R. Tanaka, *J. Org. Chem.*, 2010, **75**, 4146–4153.
185 A. Orellana and T. Rovis, *Chem. Commun.*, 2008, 730–732.
186 S. P. Lathrop and T. Rovis, *Chem. Sci.*, 2013, **4**, 1668–1673.
187 H. Ren, R. Liu, L. Chen, T. Zhu, W. M. Zhu and Q. Q. Gu, *Arch. Pharmacal Res.*, 2010, **33**, 499–502.
188 T. Komoda, Y. Sugiyama, N. Abe, M. Imachi, H. Hirota and A. Hirota, *Tetrahedron Lett.*, 2003, **44**, 1659–1661.
189 T. Komoda, M. Kishi, N. Abe, Y. Sugiyama and A. Hirota, *Biosci., Biotechnol., Biochem.*, 2004, **68**, 903–908.
190 T. Komoda, Y. Sugiyama, N. Abe, M. Imachi, H. Hirota, H. Koshino and A. Hirota, *Tetrahedron Lett.*, 2003, **44**, 7417–7419.
191 X. Wang and J. A. Porco Jr, *Angew. Chem., Int. Ed.*, 2005, **44**, 3067–3071.
192 C. Li, X. Li and R. Hong, *Org. Lett.*, 2009, **11**, 4036–4039.
193 A. P. Marcus and R. Sarpong, *Org. Lett.*, 2010, **12**, 4560–4563.
194 G. Lang, A. L. Cole, J. W. Blunt and M. H. G. Munro, *J. Nat. Prod.*, 2006, **69**, 151–153.
195 T. Hosoe, K. Fukushima, K. Takizawa, T. Itabashi, K. Yoza and K. Kawai, *Heterocycles*, 2006, **68**, 1949–1953.
196 C. Kasettrathat, N. Ngamrojanavanich, S. Wiyakrutta, C. Mahidol, S. Ruchirawat and P. Kittakoop, *Phytochemistry*, 2008, **69**, 2621–2626.
197 M. Leyte-Lugo, M. González-Andrade, M. del C. González, A. E. Glenn, C. M. Cerda-García-Rojas and R. Mata, *J. Nat. Prod.*, 2012, **75**, 1571–1577.
198 J. Li, S. Liu, S. Niu, W. Zhuang and Y. Che, *J. Nat. Prod.*, 2009, **72**, 2184–2187.
199 G. Y. Chen, H. Huang, J. L. Ye, A. E. Wang, H. Y. Huang, H. K. Zhang and P. Q. Huang, *Chem.–Asian J.*, 2012, **7**, 504–518.
200 R. Nakai, H. Ogawa, A. Asai, K. Ando, T. Agatsuma, S. Matsumiya, S. Akinaga, Y. Yamashita and T. Mizukami, *J. Antibiot.*, 2000, **53**, 294–296.
201 T. Agatsuma, T. Akama, S. Nara, S. Matsumiya, R. Nakai, H. Ogawa, S. Otaki, S. Ikeda, Y. Saitoh and Y. Kanda, *Org. Lett.*, 2002, **4**, 4387–4390.
202 T. H. Lambert and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2006, **128**, 426–427.
203 R. Nakai, H. Ishida, A. Asai, H. Ogawa, Y. Yamamoto, H. Kawasaki, S. Akinaga, T. Mizukami and Y. Yamashita, *Chem. Biol.*, 2006, **13**, 183–190.
204 J.-H. Jang, Y. Asami, J.-P. Jang, S.-O. Kim, D. O. Moon, K.-S. Shin, D. Hashizume, M. Muroi, T. Saito, H. Oh, B. Y. Kim, H. Osada and J. S. Ahn, *J. Am. Chem. Soc.*, 2011, **133**, 6865–6867.
205 J. Deng, B. Zhu, Z. Lu, H. Yu and A. Li, *J. Am. Chem. Soc.*, 2012, **134**, 920–923.
206 J. Xu, E. J. Caro-Diaz, L. Trzoss and E. A. Theodorakis, *J. Am. Chem. Soc.*, 2012, **134**, 5072–5075.
207 J. Yin, C. Wang, L. Kong, S. Cai and S. Gao, *Angew. Chem., Int. Ed.*, 2012, **51**, 7786–7789.

208 K. N. Maloney, W. Hao, J. Xu, J. Gibbons, J. Hucul, D. Roll, S. F. Brady, F. C. Schroeder and J. Clardy, *Org. Lett.*, 2006, **8**, 4067–4070.
209 A. Chatzimpaloglou, M. P. Yavropoulou, K. E. Rooij, R. Biedermann, U. Mueller, S. Kaskel and V. Sarli, *J. Org. Chem.*, 2012, **77**, 9659–9667.
210 K. Kobayashi, I. Okamoto, N. Morita, T. Kiyotani and O. Tamura, *Org. Biomol. Chem.*, 2011, **9**, 5825–5832.
211 J. Hiort, K. Maksimenka, M. Reichert, S. Perovic-Ottstadt, W. Lin, V. Wray, K. Steube, K. Schaumann, H. Weber, P. Proksch, R. Ebel, W. E. G. Muller and G. Bringman, *J. Nat. Prod.*, 2004, **67**, 1532–1543.
212 G. Schlingmann, T. Taniguchi, H. He, R. Bigelis, H. Y. Yang, F. E. Koehn, G. T. Carter and N. Berova, *J. Nat. Prod.*, 2007, **70**, 1180–1187.
213 Y. Miyake, C. Ito, M. Itoigawa and T. Osawa, *Biosci., Biotechnol., Biochem.*, 2007, **71**, 2515–2521.
214 Y. Miyake, M. Mochizuki, C. Ito, M. Itoigawa and T. Osawa, *Biosci., Biotechnol., Biochem.*, 2008, **72**, 1580–1585.
215 A. J. Kim, J. N. Choi, J. Kim, H. Y. Kim, S. B. Park, J. H. Choi, K. H. Liu and C. H. Lee, *J. Microbiol. Biotechnol.*, 2012, **22**, 100–106.
216 R. Riko, H. Nakamura and K. Shindo, *J. Antibiot.*, 2014, **67**, 179–181.
217 Y. Miyake, C. Ito, H. Tokuda, T. Osawa and M. Itoigawa, *Biosci., Biotechnol., Biochem.*, 2010, **74**, 1120–1122.
218 M. Isaka, P. Chinthanom, P. Rachtawee, W. Somyong, J. J. Luangsa-ard and N. L. Hywel-Jones, *Phytochem. Lett.*, 2013, **6**, 162–164.
219 P. Sauleau, P. Retailleau, J. Vacelet and M.-L. Bourguet-Kondracki, *Tetrahedron*, 2005, **61**, 955–963.
220 T. Gebreyesusa, T. Yosief, S. Carmely and Y. Kashmanb, *Tetrahedron Lett.*, 1988, **29**, 3863–3864.
221 M. Tsuda, T. Mugishima, K. Komatsu, T. Sone, M. Tanaka, Y. Mikami, M. Shiro, M. Hirai, Y. Ohizumi and J. Kobayashi, *Tetrahedron*, 2003, **59**, 3227–3230.
222 E. Brück, A. Elbert, R. Fischer, S. Krueger, J. Kühnhold, A. M. Klueken, R. Nauen, J.-F. Niebes, U. Reckmann and H.-J. Schnorbach, *Crop Prot.*, 2009, **28**, 838–844.