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REVIEW ARTICLE

The unique chemistry and biology of the piericidins

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The piericidin family of microbial metabolites features a 4-pyridinol core linked with a methylated polyketide side chain. Piericidins are exclusively produced by actinomycetes, especially members of the genus *Streptomyces*. The close structural similarity with coenzyme Q renders the piericidins important NADH-ubiquinone oxidoreductase (complex I) inhibitors in the mitochondrial electron transport chain. Because of the significant activities of the piericidins, which include insecticidal, antimicrobial and antitumor effects, total syntheses of the piericidins were developed using various synthetic strategies. The biosynthetic origin of this class has also been the subject of investigation. This review covers the isolation and structure determination of the natural piericidins, their chemical modification, the total syntheses of natural and unnatural analogs, their biosynthesis, and reported biological activities together with structure–activity relationships. Given the fundamental biology of this class of metabolites, the piericidin family will likely continue to attract attention as biological probes of important biosynthetic processes.
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INTRODUCTION

The piericidins are a family of structurally related compounds isolated from actinomycetes, especially from members of the genus *Streptomyces*. The striking structural resemblance between piericidins (such as piericidin A, **1**) and coenzyme Q (**2**), shown in Figure 1, suggested that piericidins act as coenzyme Q antagonists. This has been confirmed by the finding that piericidins are specific and potent NADH-ubiquinone oxidoreductase (complex I) inhibitors in the mitochondrial electron transport chain.[1] Yoshida and Takahashi[2] reviewed the piericidins in 1978, before the full complexity of this class was known.

With the development of new chemical diversity and enhanced biological studies of the piericidins during the past four decades, this unique class is showing potential value in cell biology studies, although the toxicities to mammals are still inescapable. In this review, we examine the up-to-date literature on both natural and synthetic piericidins, and include more recent studies defining their total syntheses and biosynthesis. Information on bioactivities and structure–activity relationships (SAR) is also discussed.

The Origins of the Piericidins

Piericidin A (also named piericidin A1 in some references) was reported by Takahashi and co-workers as a new insecticidal metabolite produced by cultures of the soil-derived actinomycete *Streptomyces mobaraensis*. The original reports included details of its isolation, physiological activity, functional groups and a partial structure.[3–5] The structure of piericidin A was originally proposed in 1965 as **1a** (Figure 1) on the basis of extensive degradation studies and analysis of the ^{1}H NMR data.[6] Piericidin A resembles coenzyme Q in its overall structure containing a pyridine ring with two adjacent methoxy groups, rather than the identically substituted *p*-benzoquinone functionality.[7] The structure of the pyridine ring was subsequently confirmed by synthesis,[8] and the configurations of the asymmetric centers, C-9 and C-10, were proposed to be *S* in 1967.[9,10]

Ultimately, by a combination of ^{13}C-NMR spectral analyses and ^{13}C feeding experiments, the structure of piericidin A (**1**) was revised by reassigning the C-4–C-5 olefin to C-5–C-6. Further, in 1977, the same group confirmed their assignment by selective reduction and the application of mass spectral analysis.[11,12] Then, in 1983, the configurations of C-9 and C-10 were revised to 9*R*, 10*R* on the basis of strong optical rotation data.[13] The full stereochemistry of the side chain was also confirmed by enantioselective synthesis several years later yielding solid proof of the structure of piericidin A (**1**).[14,15]

Natural piericidin aglycones

Over time, numerous new piericidin analogs were discovered. Piericidin B (**3**, also named piericidin B1 in some references) was also originally observed in the cultivation of *Streptomyces mobaraensis* in 1963.[3] Piericidin B was assigned as the C-10 methoxy analog of A, apparently produced during fermentation and work-up.[9]

Sometime later, Takahashi reported that 14 new piericidins could be extracted from the cultured mycelium of *Streptomyces pactum*.[16] These piericidins were classified into four groups, piericidins A (**1**), A2–A4 (**4**–**6**), B (**3**), B2–B4 (**7**–**9**), C1–C4 (**10**–**13**) and D1–D4 (**14**–**17**), according to their chromatographic behavior, and their structures were assigned on the basis of mass, ^{1}H and ^{13}C NMR

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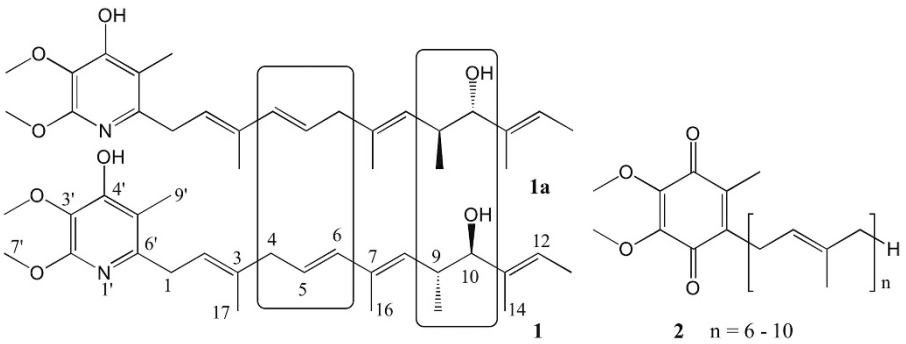

Figure 1 Structure of piericidin A (**1**) and the coenzyme Q complex (**2**).

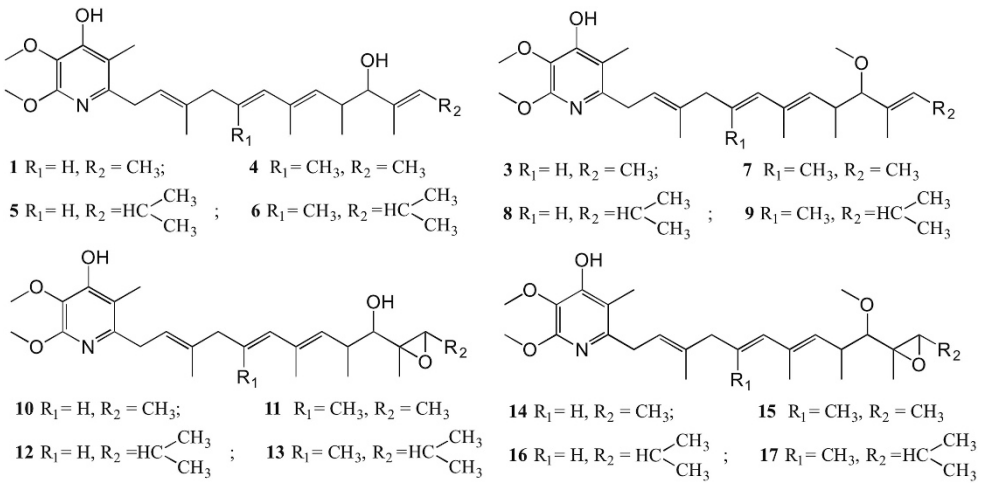

Figure 2 Structures of piericidin aglycones **1** and **3**–**17**.

spectral analyses (Figure 2).[16] The UV spectra of these piericidins are classified into two types: one, due to piericidins having an odd suffix, shows λ_{max} at 239 nm, and the other, due to even suffixed piericidins, shows λ_{max} at 225 nm.[17]

Two *N*-oxide derivatives of piericidin, piericidin B1 *N*-oxide (**18**) and piericidin B5 *N*-oxide (**19**), together with piericidin B5 (**20**), were isolated from cultures of *Streptomyces* strain MJ288-OF3. The structures of the new *N*-oxides were determined by NMR and high-resolution MS analyses and confirmed by zinc powder reduction to yield piericidin B (**3**) and piericidin B5 (**20**).[18,19]

Other piericidin aglycones that include Mer-A2026 A (**21**) and B (**22**), which lack the methoxy group at C-3′ (numbered as C-5′ in the original reference), were isolated from cultures of *Streptomyces pactum* Me2108. Two new piericidins, IT-143-A (**23**) and B (**24**) were isolated from the culture mycelia of *Streptomyces* sp. IT-143.[20] A new member of the piericidin family, JBIR-02 (**25**), was isolated from the mycelium of *Streptomyces* sp. ML55, together with two known piericidin derivatives, piericidin A (**1**) and IT-143-B (**24**).[21] NMR analysis, including NOE experiments, showed that **25** was a 3:1 tautomeric mixture of pyridone (**25a**) and hydroxypyridine (**25b**). The absolute configurations of **22** (9*R*,10*R*) and **25** (9*R*,10*R*) were subsequently established by total synthesis.[22]

Piericidin C5 (**26**), together with piericidins C1 (**10**), C3 (**12**), D1 (**14**) and A3 (**5**), were isolated from the fermentation broth of a *Streptomyces* sp., whereas piericidin C6 (**27**), as well as piericidins C2 (**11**) and C4 (**13**) were isolated from the culture of a *Nocardioides* species. This is the first report of piericidins produced by a microorganism other than members of the genus *Streptomyces*.[23] Piericidins C7 (**28**) and C8 (**29**) were found to be produced by a *Streptomyces* sp., associated with a marine ascidian.[24,25] 7-Demethylpiericidin A1 (**30**) was isolated from a soil-derived *Streptomyces* sp. SN-198.[26] A deep sea-derived *Streptomyces* sp. SCSIO 03032, yielded piericidin E1 (**31**) a novel analog featuring a C-2/C-3 epoxide ring (Figure 3).[27]

Natural piericidin glycosides

The first piericidin glycosides, glucopiericidins A (**32**) and B (**33**) were reported in 1987. These two glycosides, defined as piericidin A, 10-*O*-*β*-D-glucoside (glucopiericidin A, **32**) and piericidin A, 4′-*O*-*β*-D-glucoside (glucopiericidin B, **33**), were isolated from the culture broth of the soil-derived *Streptomyces pactum* S48727, together with the aglycone piericidin A (**1**).[28] In the cultivation, glucopiericidin A (**32**) was produced after the appearance of piericidin A (**1**), indicating that glucopiericidin A (**32**) was produced by glucosylation of piericidin A (**1**). Glucopiericidin A (**32**) was a main product after 96-h incubation, whereas piericidin A (**1**) and glucopiericidin B (**33**) were minor.[28]

Another two new piericidin glycosides, glucopiericidinols A1 (**34**) and A2 (**35**), possessing an additional hydroxy group at C-3, were isolated from the cultured broth of *Streptomyces* sp. OM-5689.[29] In the same strain, another glycoside called 13-hydroxyglucopiericidin A (**36**) was reported in the next year.[30] Two new piericidin rhamnosides, 4′-rhamnopiericidin A1 (**37**) and 7-demethyl-4′-rhamnopiericidin A1 (**38**), were isolated from the soil *Streptomyces* sp. SN-198 and reported

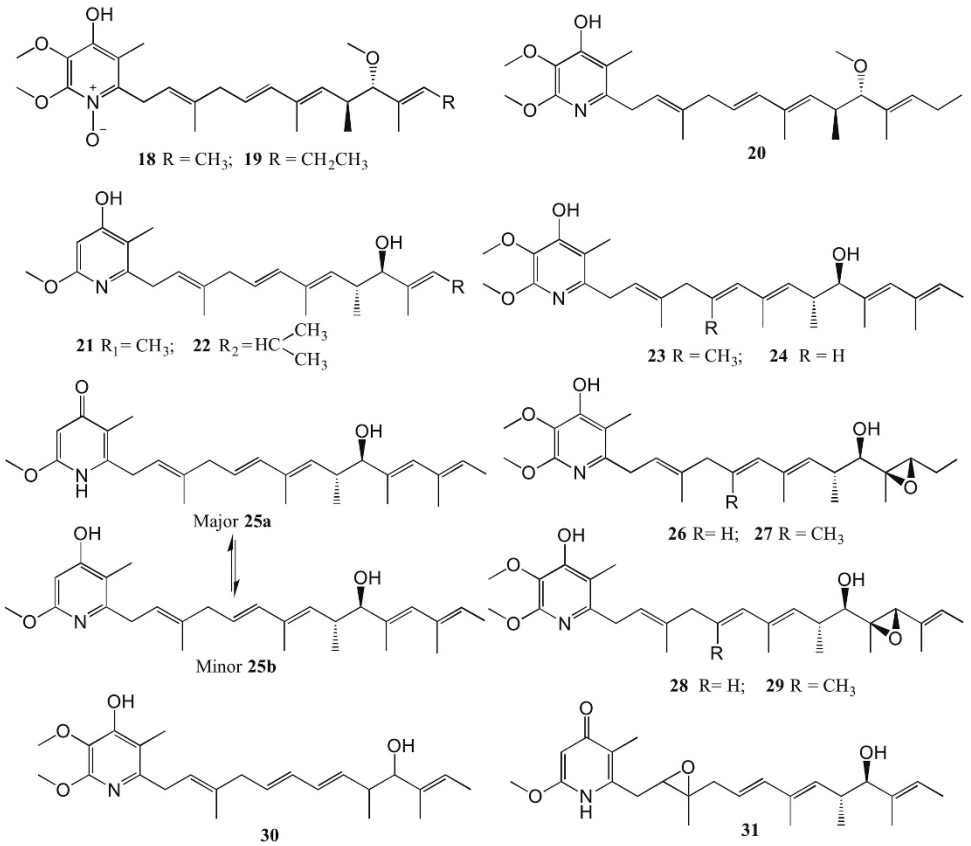

Figure 3 Structures of piericidin aglycones **18**–**31**.

in 1990 and 1996, respectively.[26,31] In the mycelial extract of *Streptomyces* sp. DO-100, isolated from a soil sample, a piericidin glycoside with an unusual methylhexose moiety was discovered, and its structure was determined to be 4′-deoxytalopiericidin A1 (**39**) by one-dimensional NMR and NOE experiments.[32] A new piericidin glycoside derivative named glucopiericidin C (**40**) was isolated, together with glucopiericidin A and piericidin A, from a marine sediment-derived *Streptomyces* isolate, B8112. The structure of glucopiericidin C (**40**) was assigned as the 10-*O*-*β*-D-glucoside of Mer-A2026A. Glucoside **40** was similar to glucopiericidin A, except for the loss of the methoxy group at C-3′ (Figure 4).[33]

CHEMICAL MODIFICATION AND TOTAL SYNTHESIS

Synthetic piericidin analogues

The rare structure of piericidin A consists of a 2,3-dimethoxy-5-methyl-4-pyridinol ring bonded at C-6 with a poly-unsaturated and -methylated side chain. Schmidtchen and Rapoport[34] prepared the pyridinol ring and attached conventional polyisoprenoid side chains for structure–activity studies. The synthetic piericidin analogues (**41a–e**) were tested for their activities of complex I inhibition. The farnesyl analogue **41c** illustrated potent inhibitory effects, with similar activity to piericidin A (**1**). Surprisingly, the solanesyl analogue **41e** was two orders of magnitude less active.[34]

Takahashi *et al.*[35] also synthesized various piericidin analogues to clarify the respiratory inhibition relationships (SAR) of piericidins and their analogs.[36] Finally, 12 analogues (**42a–d**, **43a–d** and **44a–d**), comprising three series depending on the ring structures, were synthesized by a Wittig reaction, as well as their saturated side-chain analogues (**45a–d**, **46a–d** and **47a–d**).[35]

The diacetate **48** and methyl ether **49** derivatives of Mer-A2026A (**21**, 3′-demethoxyl piericidin A1) were prepared as part of the structure determination of **21**.[37] Two piericidin analogues, **50** and **51**, having a simple benzene ring were synthesized by a palladium-catalyzed cross-coupling reaction and the reaction of sulfone and aldehyde using the Julia coupling procedure.[38] Analog **50** with a 2*E* olefin configuration has the same side chain as natural piericidin (piericidin B, **3**) and **51** is the 2*Z*-isomer of **50** at the side chain (Figure 5).

Following the successful completion of the total synthesis of piericidin A (**1**) and B1 (**3**), Boger and co-workers also prepared a series of key analogues.[39] *Ent*-Piericidin A1 (**52**) and the C-10 hydroxy diastereomer (**53**) were prepared from the pivalate ester used as an intermediate in the total synthesis of **3** and its ketone analog **41**. The C-4′-acetate, C-10 ketone **54** was prepared from the alcohol **55**, which was derived from piericidin A (**1**). Mild acetate hydrolysis of **54** provided the C-10 ketone **56**. The C-4′ methyl ethers **57** and **58** were prepared by selective methylation of **1** and **41c**, another analog they also obtained. More significantly, C-4′-deshydroxypiericidin A1 (**59**) and the C-4′-deshydroxy analogue (**60**) were prepared by total synthesis for biological examination.

Boger and co-workers were also successful in accessing the C-5′-desmethyl and C-4′-deshydroxy–C-5′-desmethyl analogues bearing the natural piericidin side chain as well as the simplified farnesyl side chain, 5′-desmethylpiericidin A1 (**61**), 4′-deshydroxy-5′-desmethylpiericidin A1 (**62**) and the corresponding farnesyl side-chain bearing analogues (**63**, **64**; Figure 6).

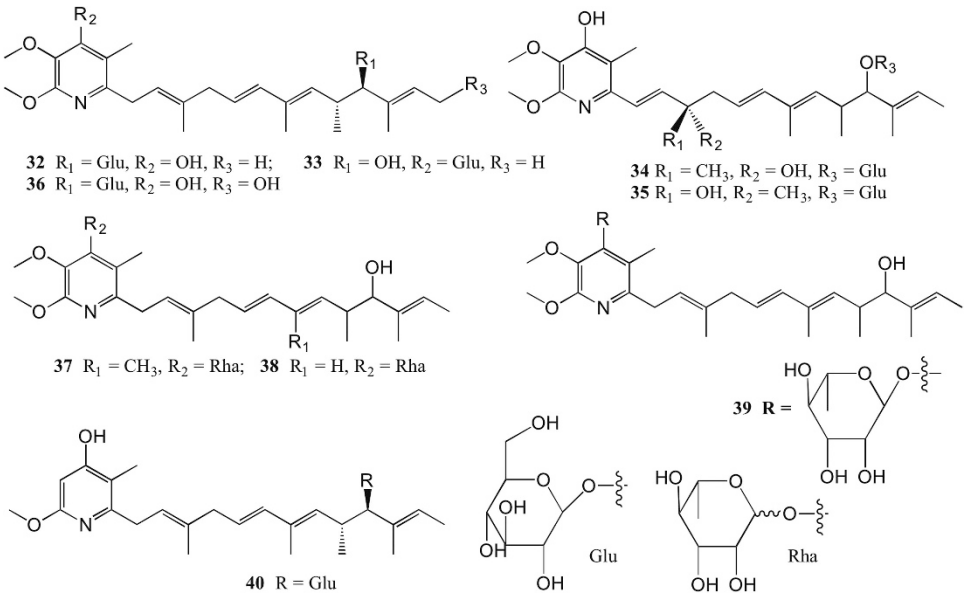

Figure 4 Structures of piericidin glycosides **32**–**40**.

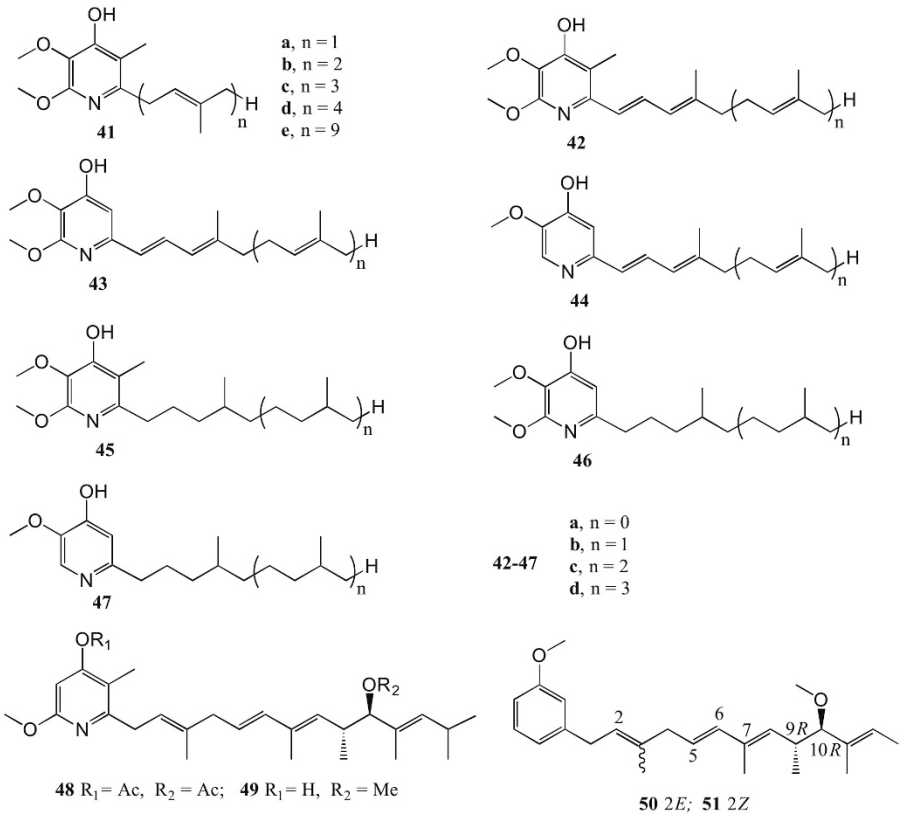

Figure 5 Structures of synthetic piericidin analogues **41**–**51**.

Total synthesis of piericidins

Although the preparation of the fully elaborated pyridine ring system substituted with simplified side chains was reported in 1977,[34] as well as an asymmetric synthesis of the C-6–C-13 segment of the side chain in 1991,[15] there were no total syntheses of piericidins until 2005. Boger and co-workers reported the first total synthesis of piericidin A (**1**) and B1 (**3**) in 2005 in an early paper,[40] and then provided full details in the next year.[39] Shortly following Boger's preliminary disclosure, Keaton and Phillips[41] reported a complementary total synthesis of 7-demethylpiericidin A1 (**30**), a natural product closely related to piericidin A (**1**). In contrast to Boger's method, Akita *et al.*[42,43] reported a new total synthesis, in 2009, of **1** and **3** based on a convergent route involving a Julia coupling strategy. Lipshutz and Amorelli[44] also described a unique strategy for the key disconnection, highlighting a modified Negishi carboalumination/Ni-catalyzed cross-coupling on a polyenyne precursor. Subsequently, Gademann

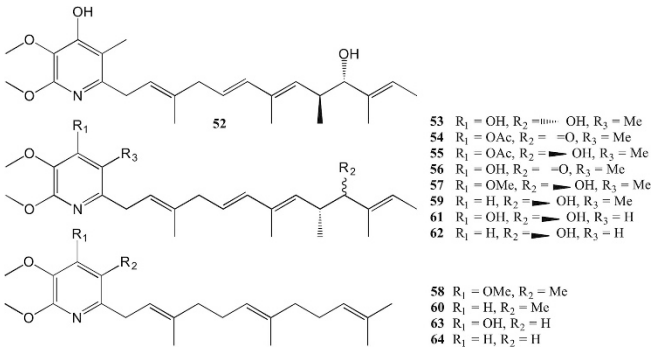

Figure 6 Structures of synthetic piericidin analogues **52**–**64**.

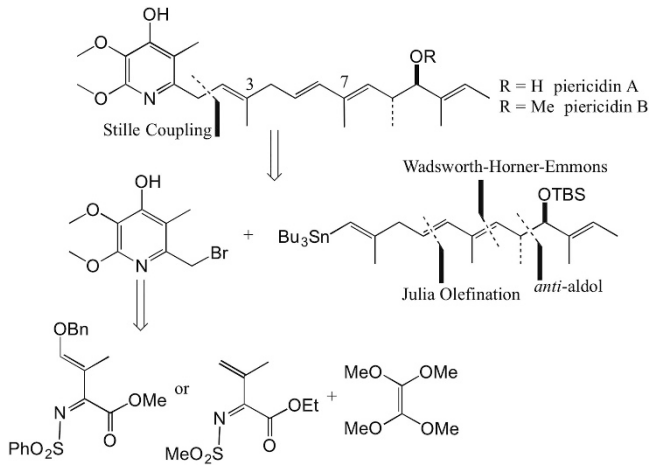

Figure 7 Boger's synthetic strategy for piericidins A (**1**) and B1 (**3**).

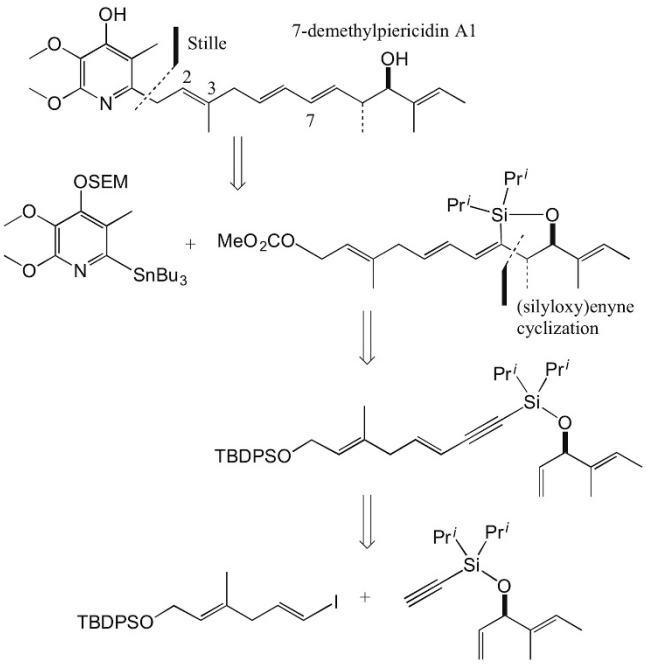

Figure 8 Phillips' synthetic strategy for 7-demethylpiericidin A1 (**30**).

and co-workers reported the total syntheses of the piericidin-related natural products Mer-A2026B (**22**) and JBIR-02 (**25**).[22]

Central to Boger's approach is an inverse electron demand Diels–Alder reaction between an *N*-sulfonyl-1-aza-1,3-butadiene and tetramethoxyethene followed by Lewis acid promoted aromatization to assemble the functionalized pyridine core. Additional key elements in the convergent approach include the use of an asymmetric anti-aldol reaction to install the relative and absolute stereochemistry at C-9 and C-10, a modified Julia Olefination for formation of the C-5–C-6 *trans*-double bond with convergent assemblage of the side chain, and a penultimate heterobenzylic Stille cross-coupling reaction of the pyridyl core with the fully elaborated side chain (Figure 7).[39,40]

Shortly after Boger's report on the synthesis of piericidin A (**1**) and B1 (**3**), Phillips and Keaton[41] reported a convergent total synthesis of 7-demethylpiericidin A1 (**30**) that is patterned on the strategy outlined in Figure 8. The total synthesis proceeded in nine steps from Tiglic aldehyde, and features an application of Titanium (II)-mediated cyclization of a (silyloxy)enyne as the key reaction for the assembly of the polyene domain.[41]

Akita *et al.* reported the synthesis of a (±)-piericidin B (**3**) analog possessing a benzene ring instead of pyridine ring in 1997.[45] Further, in 2009, they reported a new total synthesis of piericidin A (**1**) and B1 (**3**) based on a convergent synthesis via Julia coupling different than Boger's method, which is based on carbon–carbon bond formation by Stille cross-coupling and the construction of the two stereogenic centers in the side chain by the use of an asymmetric anti-aldol reaction.[39,40] Akita's synthetic plan for **1** and **3** was based on double bond formation between the left and right halves as shown in Figure 9. The construction of the two stereogenic centers in the right half could be achieved by lipase-catalyzed enantioselective hydrolysis of α-methyl-β-acetoxy ester. The non-conjugated aldehyde left half corresponding to the C-1–C-5 unit of the piericidin side chain was prepared starting from the known allyl alcohol congener.[42,43]

Lipschitz's and Amorelli's approach to a practical synthesis of piericidin A (**1**) highlights a modified Negishi carboalumination followed by a Ni-catalyzed cross-coupling strategy. This strategy allows for couplings of benzylic chlorides and *in situ* generated vinylalanes, arrived at via stereoselective carboalumination of terminal alkynes. Retrosynthetically, the key disconnection features a penultimate one-pot Ni-catalyzed coupling of vinylalane (right half), generated *in situ* via a modified carboalumination, to the chloromethylated pyridine (left half, Figure 10). The skipped enyne is anticipated by a propargyl selective (over allenyl) coupling of a corresponding vinyl iodide and TMS-propyne. A vinylogous Mukaiyama aldol reaction generated the eight-carbon vicinal methyl/hydroxyl side chain framework.[44] This route, to the synthesis of piericidin A (**1**) in a total of 18 steps from

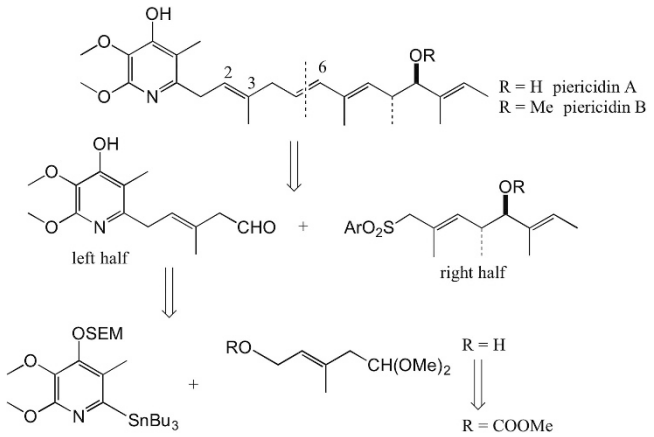

Figure 9 Akita's synthetic strategy for piericidins A (**1**) and B1 (**3**).

commercial material, involves a longest linear sequence of 11 steps from the *N*,*O*-silyl ketene acetal (Figure 10), which overall compares very favorably with the previous synthesis of piericidin A (**1**).[39–41]

The total syntheses of the piericidin related natural products Mer-A2026B (**22**) and JBIR-02 (**25**) were reported in 2013 by Gademann's group.[22] The total synthesis started with an Ir-catalyzed one-pot C–H activation reaction in combination with an oxidation procedure for the preparation of the protected bromopyridinol in five steps. The preparation of the side chain was also started from the *N*,*O*-silyl ketene acetal by a highly diastereoselective vinylogous Mukaiyama aldol reaction. The final connection of both moieties, the 4-hydroxypyridine core and the fully elaborated polyene chain, was realized after extensive experimentation with the Negishi cross-coupling reaction without isomerization of the labile side chain. Furthermore, this strategy opens up the stage for the synthesis of a number of piericidins by simply changing the coupling partner for the Horner–Wadsworth–Emmons reaction or the organometallic species for the cross-coupling reaction. In brief, key features of the synthetic approach involved a C–H activation/oxidation procedure for the preparation of the highly functionalized pyridine moiety, a vinylogous Mukaiyama aldol reaction, and a crucial Negishi cross-coupling of an advanced pyridine derivative with an allylic side-chain precursor (Figure 11).[22]

BIOSYNTHSIS OF PIERICIDIN A

In 1969, Takahashi and co-workers[46] had proven by degradation studies that the long-branched C23 chain of the piericidins (**65** in Figure 12) was formed from five propionate and four acetate units, and that a nitrogen atom was introduced at the terminal part of the chain, followed by cyclization to form the pyridine ring. The two methoxyl groups on the pyridine rings of the piericidins were derived from *S*-methylmethionine.[46] This conclusion had been confirmed by Tanabe and Seto[47] in the next year by NMR measurements of satellite bands due to sp^3-carbons, which were readily assigned by a selective ^{13}C–^{1}H decoupling technique. Although the total synthesis of piericidin A has been achieved successfully, studies on the biosynthesis of piericidins were limited before 2012.

In 2012, the You and Tang groups provided an overview of the biosynthetic pathway for piericidin A (**1**; Figure 13).[48] Sequential analysis of the *Streptomyces piomogeues* genome revealed six modular polyketide synthases, an amidotransferase, two methyltransferases and a monooxygenase responsible for the production of **1**. Gene functional analysis and deletion studies revealed with confidence that **1** originates

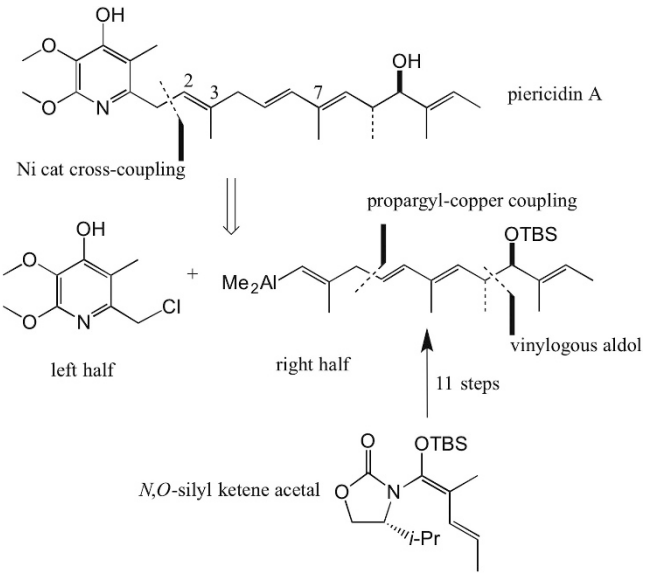

Figure 10 Lipschutz's synthetic strategy for piericidin A (**1**).

from a modular polyketide synthase pathway.[48] Unexpectedly, the nitrogen atom in piericidin A was found to be a result of an ATP-dependent amidotransferase rather than the more common hybrid polyketide synthase–non ribosomal peptide synthetase pathway.[48]

The piericidin A (**1**) gene cluster was also identified from the deep-sea-derived *Streptomyces* sp. SCSIO 03032 by Zhang and colleagues in 2014.[27] *In vivo* and *in vitro* experiments verified *PieE* as a 4′-hydroxylase and *PieB2* as a 4′-*O*-methyltransferase (*sic.*, the C number 4′ is according to the original reference), allowing the elucidation of the post-polyketide synthase modification steps involved in piericidin A biosynthesis (Figure 14).[27]

PIERICIDIN BIOACTIVITIES

Complex I inhibitory activities and SAR studies

Piericidin A (**1**) is well known as an effective inhibitor of the electron transport chain, also called the respiratory chain.[49,50] There are four protein complexes, labeled complex I–IV, in the electron transport chain, which are involved in moving electrons from NADH and succinate to molecular oxygen. Piericidin A (**1**) is an especially potent inhibitor of complex I that bind to its ubiquinone binding site,[51–53] with a K_i ranging between 0.6[54] and 1 nM.[55] Respiratory inhibition by piericidin A (**1**) can be reversed by the addition of vitamin-K3 (menadione) to the inhibited respiratory chain in mammalian mitochondria, but not in insect mitochondria.[56]

SAR studies of complex I inhibitors are important not only to elucidate the structural factors required for their inhibitory action but also to determine the structural properties of the ubiquinone reduction site in the enzyme. Natural piericidins, as well as synthetic analogues, were examined in SAR studies by Takahashi and co-workers with mammalian and insect mitochondria, in and before 1980.[6,11,16,35,36] Several review papers and book chapters also cited their results.[36,57–59] In 2006, Boger also reported detailed SAR studies using synthetic piericidin analogues measuring their complex I inhibitory activities.[39]

On the basis of SAR studies of a number of synthetic piericidin analogues possessing different side chains, it was concluded that a branched methyl group at C-3 and unsaturation between C-2 and C-3 are important for complex I inhibitory activity.[35,36] It was considered

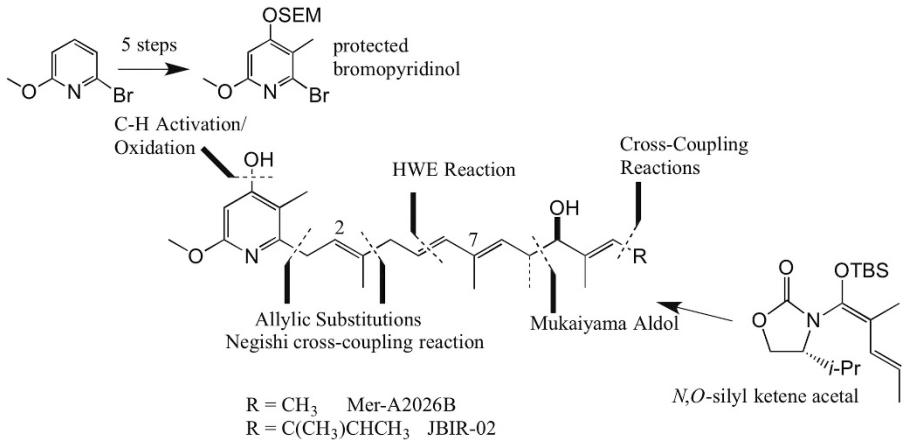

Figure 11 Key features of Gademann's total syntheses of Mer-A2026B (**22**) and JBIR-02 (**25**).

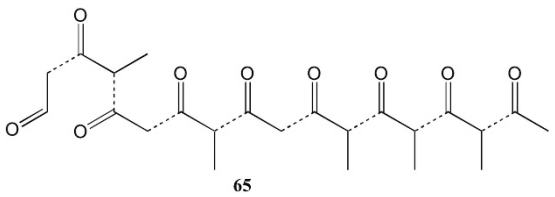

Figure 12 Long-branched C23 chain (**65**) in the biosynthesis of piericidins.

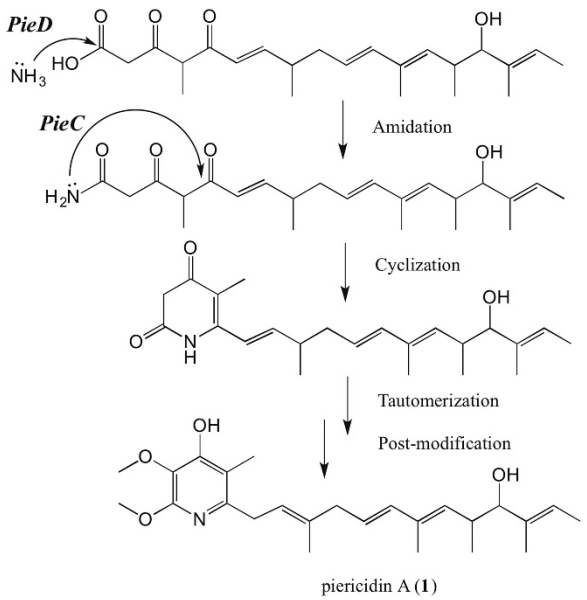

Figure 13 Proposed biosynthetic pathway of piericidin A (**1**).[48]

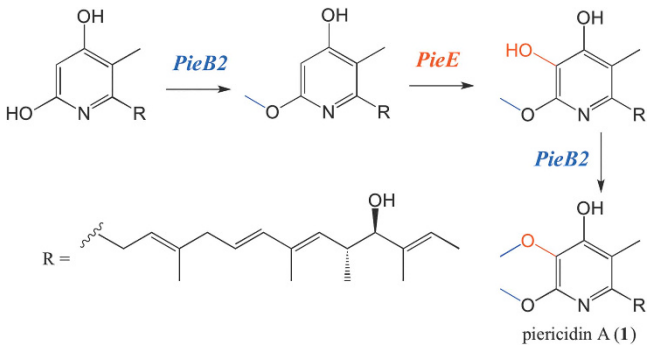

Figure 14 Genetic organization of the pie gene cluster in *Streptomyces* sp. SCSIO 03032 and the proposed piericidin A (**1**) biosynthetic pathway.[27]

that the side chain might serve primarily to enhance the hydrophobicity of the whole structure, as derivatives possessing a simple long alkyl chain retained fairly potent activity.[60] However, the location of the hydrophobic side chain at either the C-5′ or C-6′ position of the pyridine ring does not affect the inhibitory potency.[36,60] The phenolic hydroxy group on the pyridine ring was seen as necessary to maintain the high level of activity.[61] Takahashi and Yoshida synthesized a variety of piericidin analogues to elucidate the contributions of the C-2′, C-3′ and C-5′ functional groups on the pyridine ring, but their assay results were somewhat confusing.[35,36] The Boger group examined 15 synthetic piericidin analogues (**41a**, **41c** and **52–64**), together with natural piericidin A (**1**), B1 (**3**) and rotenone, for inhibition of complex I.[39] The results showed that small modifications in the side chain had little impact on this activity; *O*-methylation of the C-10 hydroxy group (**3**, half-maximal inhibitory concentration (IC_{50}) 5.1 nM) with piericidin A (**1**, IC_{50} 3.7 nM), its conversion to a ketone (**56**, IC_{50} 6.3 nM), and inversion of the C-10-OH configuration (**53,** IC_{50} 8 nM) resulted in very small changes in potency. The piericidin analog with the simplified farnesyl side chain (**41c**, IC_{50} 3.8 nM) was similar in potency to **1**. However, the overriding importance of the side chain became quite clear in examining **41a** (IC_{50} 10 000 nM) in which removal of the C-5–C-16 segment resulted in an extreme loss in activity (Figure 15).

The pyridyl C-4′ hydroxyl group also proved to be important for the inhibition of complex I. In acetylated analogs (**54**, IC_{50} 26 nM; **55**, IC_{50} 24 nM) or methylated analogs (**57**, IC_{50} 130 nM), the transformation led to 7- and 35-fold reductions in potency. Removal altogether as in C4′-deshydroxypiericidin (**59**, IC_{50} 10 nM) had a surprisingly modest impact. In marked contrast, *O*-methylation of the C-4′ phenol (**58**, IC_{50} 750 nM) as well as the removal of the C-4′ phenol (**60**, IC_{50} 400 nM) in the analogues bearing the simplified farnesyl side chains reduced complex I inhibition >100-fold. These latter studies supported that the C-4′ hydroxy group is central to piericidin's activity. The C-5′ methyl group of piericidin had a similar remarkable impact on the piericidin activity. Methyl group removal as in 5′-desmethylpiericidin A1 (**61**, IC_{50} 4.0 nM) had little or no impact on complex I inhibition, whereas its removal in the analogue **63**

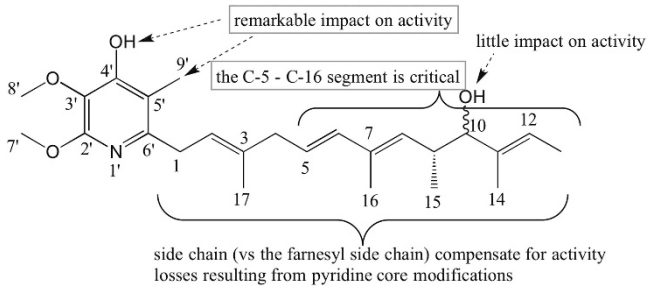

Figure 15 Brief structure–activity relationships of piericidins as complex I inhibitors. A full color version of this figure is available at *The Journal of Antibiotics* journal online.

(IC_{50} 110 nM) bearing the farnesyl side chain reduced complex I inhibition 30-fold relative to either **41c** or **1**. Just as remarkable, removing both the C-4′ hydroxyl and C-5′ methyl groups as in **62** (IC_{50} 9.0 nM) only reduced the complex I inhibition ca. 2-fold relative to piericidin A itself, whereas these modifications to yield analogue **64,** bearing the farnesyl side chain, resulted in a compound that was >500-fold less potent than **1** or **41c**. Surprisingly, the potent activity of **59, 61** and especially **62** suggests that the natural product side-chain composition (versus the farnesyl side chain) somehow compensates for losses in binding affinity that result from modifications of the pyridine core (Figure 15).

Boger's SAR studies showed that the pyridine C-4′ hydroxy group and the C-5′ methyl substituent appear more central to the potency of the piericidins, as their modification or removal leads to reductions in complex I inhibition. Although the side-chain substituents of piericidin A may impact complex I inhibition, this is most significant when key structural features of the pyridine core are removed.

Functions as Complex I inhibitors

Piericidins were first discovered because of their insecticidal activity,[3,62] and mechanistic studies indicated that complex I was the molecular target of piericidins.[63] Besides its well-known redox role in the electron transport chain, complex I is also considered to be one of the main production sites of reactive oxygen species (ROS). Complex I inhibitors can be grouped into two classes depend on their effect in the production of ROS. Piericidin A (**1**), as well as other class A inhibitors such as rotenone, increase ROS production, whereas class B inhibitors (such as stigmatellin, mucidin and capsaicin) appear to directly prevent oxygen reduction presumably acting on the electron escape site.[64] In forward electron transfer in complex I, slow superoxide production in the presence of glutamate and malate was enhanced by both rotenone and piericidin A (**1**).[65] Co-exposure of hepatocytes to isoniazid, an anti-tuberculosis drug associated with idiosyncratic liver injury, and nontoxic concentrations of **1** (30 nM) resulted in massive ATP depletion and cell death, indicating that piericidin A (**1**) triggers isoniazid-induced hepatocellular injury.[66]

The effect of piericidin A (**1**) on the sites of inhibition in and around photosystem II was examined, and the results suggested that it binds to cytochrome b_6/f complex, out of the photosystem II complex.[67] Although mitochondrial complex I inhibition has been linked to sporadic tauopathies, piericidin A (**1**) was studied to determine whether there is a pathogenic interaction with the P301S mutation. The results showed that **1** significantly increased the number of phospho-tau immunoreactive cells in the cerebral cortex in P301S mice. Furthermore, **1** led to increased levels of pathologically phosphorylated tau only in P301S mice.[68] Mitochondrial complex I dysfunction is regarded as underlying dopamine neuron death in Parkinson's disease models. However, piericidin A (**1**) as a complex I inhibitor did not induce selective dopamine neuron death.[69]

Cytotoxic and antimicrobial effects with SAR studies

Piericidin A (**1**) showed significant cytotoxic activities against several tumor cells, such as mouse leukemia cell line (L1210, IC_{50} 5 nM),[39] and was selectively cytotoxic toward human multiple myeloma cells.[70] Because of the toxicity of **1** toward cultured bioassay cells, other activities, such as antiviral activity, were not significant or not found.[71] In the acute toxicity tests administering piericidin A (**1**) by i.v. administration in mice, the LD_{50} value was $<1.0\ mg\ kg^{-1}$,[28,72] whereas glucopiericidins A (**32**) and B (**33**) showed less toxicity with LD_{50} values between 10 and 30 $mg\ kg^{-1}$. Most of the other piericidins, including the glycosides, also showed cytotoxic activities to several cancer cell lines (Table 1).

The piericidin family exhibits an array of antimicrobial activity. The antimicrobial effects of the piericidins against various Gram-positive and Gram-negative bacteria, yeast or fungi are listed in Table 1, although the tested strains differed in their susceptibility to different piericidins. Interestingly, a complex of eight symbiont-produced piericidin derivatives from cocoon extracts of beewolf digger wasps showed that complementary action confers a potent antimicrobial defense.[73] The mixture of piericidin antibiotics strongly inhibited all test microbes, and this suggests that beewolf symbionts use a defensive strategy against bacterial and fungal pathogens that corresponds to 'combination therapy' or 'combination prophylaxis' an approach increasingly used in human medicine.[73]

Structure–cytotoxic activity relationships of the piericidin aglycones showed that modification or removal of the pyridine C-4′ hydroxy group and the C-5′ methyl substituent could significant reduce cytotoxicity. The side-chain substituents of piericidin A (**1**) enhanced cytotoxic activity, but their impact seems to be most significant if the pyridine core of **1** is intact.[39]

The biological activities of the piericidin glycosides are strongly influenced by the type and location of the sugar unit. Glucopiericidin A (**32**) inhibits the growth of Gram-positive bacteria and the rice blast fungus *Piricularia oryzae*, whereas glucopiericidin B (**33**) was more active than **32**. Glucopiericidins A-C (**32**, **33** and **40**) showed higher antimicrobial activities than piericidin A (**1**) or other glycoside analogs.[28,33] It was interesting that the glycosides 3'-rhamnopiericidin A1 (**37**) and 7-demethyl-3′-rhamnopiericidin A1 (**38**) showed lower antimicrobial activities against some fungi and Gram-negative bacteria than 7-demethylpiericidin A1 (**30**) and piericidin A (**1**). In contrast, **37** and **38** showed stronger cytotoxicity against KB and K562 cells. Among the glycosides, the presence or absence of a methyl group at C-7 did not significantly affect their biological activities.[26,31]

Glucopiericidinols A1 (**34**) and A2 (**35**) showed almost no activity against various Gram-positive and Gram-negative bacteria, yeast or some fungi, but they did have inhibitory activity against the rice blast fungus *Pyricularia oryzae*.[29] 13-Hydroxyglucopiericidin A (**36**) showed lower activity against *P. oryzae*, whereas it showed strong cytotoxicity against various tumor cells *in vitro*. It is noteworthy that **36** showed stronger activity against adriamycin-resistant P388 cells (ADM) than normal P388 cells.[30] 3′-Deoxytalopiericidin A1 (**39**) inhibited cancer cells selectively.[32] It has been suggested that the differences in these levels of potency and activity may depend on the permeability of the drug to the cell membrane.[26]

Table 1 Natural piericidins: origins and cytotoxic or/and antimicrobial activities

Name	*Cells or microorganisms (IC_{50} or MIC)*	*Origins (ref.)*
Piericidin A (A1, **1**)	KB (8.9 μg ml^{-1}), K562 (>12.5 μg ml^{-1}), RG-E1A-7 (0.20 nM), Neuro-2a (0.21 nM), L1210 (5 nM); *Micrococcus luteus* (6.25 μg ml^{-1}), *Aspergillus fumigatus* (>100 μg ml^{-1}).	*S. mobaraensis*;[3,62] *S. pactum*;[16] *S. pactum* S48727;[28] *Streptomyces* sp. IT-143;[20] *Streptomyces* sp;[24] *Streptomyces* sp. ML55[21]
Piericidin B (B1, **3**)	L1210 (6 nM); no activities against Gram-positive or Gram-negative bacteria or fungi.	*S. mobaraensis*;[3,62] *S. pactum*;[16] *Streptomyces* sp. MJ288-OF3[18]
Piericidin A2 (**4**)	RG-E1A-7 (0.47 nM), Neuro-2a (0.22 nM); *Micrococcus luteus* (6.25 μg ml^{-1}), *Aspergillus fumigatus* (25–100 μg ml^{-1}).	*S. pactum*;[16] *Streptomyces* sp. IT-143;[20] *Streptomyces* sp.[24]
Piericidins A3, C1, C3, D1 (**5**, **10**, **12**, **14**)	Fertilized starfish (*Asterina pectinifera*) eggs (0.07–0.09 μg ml^{-1}).	*S. pactum*;[16] *Streptomyces* sp.[23]
Piericidins C2, C4 (**11**, **13**)	Fertilized starfish (*Asterina pectinifera*) eggs (0.08–0.10 μg ml^{-1}).	*S. pactum*;[16] *Nocardioides* sp.[23]
Piericidins A4, B2–4, D2–4 (**6**–**9**, **15**–**17**)		*S. pactum*[16]
Piericidins B1 *N*-oxide, B5 *N*-oxide (**18**, **19**)	Several Gram-positive and Gram-negative bacteria and fungi (3.1–50 μg ml^{-1}).	*Streptomyces* sp. MJ288-OF3[18,19]
Piericidin B5 (**20**)	No activities against Gram-positive or Gram-negative bacteria or fungi.	
Mer-A2026A, B (**21**, **22**)		*S. pactum* Me2108[37]
IT-143-A (**23**)	*Micrococcus luteus* (6.25 μg ml^{-1}), *Aspergillus fumigatus* (12.5–25 μg ml^{-1}).	*Streptomyces* sp. IT-143[20]
IT-143-B (**24**)	*Micrococcus luteus* (6.25 μg ml^{-1}), *Aspergillus fumigatus* (>100 μg ml^{-1}).	*Streptomyces* sp. IT-143;[20] *Streptomyces* sp. ML55[21]
JBIR-02 (**25**)		*Streptomyces* sp. ML55[21]
Piericidin C5 (**26**)	Fertilized starfish (*Asterina pectinifera*) eggs (0.09 μg ml^{-1}).	*Streptomyces* sp.[23]
Piericidin C6 (**27**)	Fertilized starfish (*Asterina pectinifera*) eggs (0.09 μg ml^{-1}).	*Nocardioides* sp.[23]
Piericidin C7 (**28**)	RG-E1A-7 (1.5 nM), Neuro-2a (0.83 nM).	*Streptomyces* sp.[24,25]
Piericidin C8 (**29**)	RG-E1A-7 (0.45 nM), Neuro-2a (0.21 nM).	
7-Demethylpiericidin A1 (**30**)	KB (11.0 μg ml^{-1}), K562 (>12.5 μg ml^{-1}).	*Streptomyces* sp. SN-198[26]
Piericidin E1 (**31**)		*Streptomyces* sp. SCSIO 03032[27]
Glucopiericidin A (**32**)	HeLa S3 (0.25, 0.11 μg ml^{-1}), B16 (0.0074 μg ml^{-1}), H-69 (0.019 μg ml^{-1}), P388 (0.36 μg ml^{-1}), P388/ADM (0.25 μg ml^{-1}); *Pyricularia oryzae* (50, 31 μg ml^{-1}), several Gram-positive bacteria (12.5–50 μg ml^{-1})	*S. pactum* S48727[28]
Glucopiericidin B (**33**)	*Pyricularia oryzae* (25 μg ml^{-1}), several Gram-positive bacteria and fungi (12.5–50 μg ml^{-1})	
Glucopiericidinol A1 (**34**)	HeLa S3 (0.39, 0.62 μg ml^{-1}), B16 (0.32 μg ml^{-1}), H-69 (0.47 μg ml^{-1}), P388 (0.58 μg ml^{-1}), P388/ADM (4.3 μg ml^{-1}); *Pyricularia oryzae* (125 μg ml^{-1}), no activity against 14 microorganisms.	*Streptomyces* sp. OM-5689[29]
Glucopiericidinol A2 (**35**)	HeLa S3 (0.10, 0.98 μg ml^{-1}), B16 (0.67 μg ml^{-1}), H-69 (0.83 μg ml^{-1}), P388 (1.6 μg ml^{-1}), P388/ADM (4.2 μg ml^{-1}); *Pyricularia oryzae* (31 μg ml^{-1}), no activity against 14 microorganisms.	
13-Hydroxyglucopiericidin A (**36**)	HeLa S3 (0.76 μg ml^{-1}), B16 (0.21 μg ml^{-1}), H-69 (0.066 μg ml^{-1}), P388 (2.5 μg ml^{-1}), P388/ADM (0.78 μg ml^{-1}); *Pyricularia oryzae* (500 μg ml^{-1}), Nno activity against 14 microorganisms	*Streptomyces* sp. OM-5689[30]
3′-Rhamnopiericidin A1 (**37**)	HeLa (2.8 μg ml^{-1}), KB (0.74, 3.8 μg ml^{-1}), K562 (4.0 μg ml^{-1}); several Gram-negative bacteria and fungi (12.5–50 μg ml^{-1}).	*Streptomyces* sp. SN-198[26,31]
7-Demethyl-3′-rhamnopiericidin A1 (**38**)	KB (4.5 μg ml^{-1}), K562 (2.9 μg ml^{-1}).	
3′-Deoxytalopiericidin A1 (**39**)	Colon 26 (0.81 μg ml^{-1}), L1210 (7.91 μg ml^{-1}).	*Streptomyces* sp. D0-100[32]
Glucopiericidin C (**40**)	36 Human tumor cell lines (mean 2.0 μM).	*Streptomyces* sp. B8112[3]

Abbreviation: IC_{50}, half-maximal inhibitory concentration.

Anti-tumor potency

Phosphatidylinositol (PI) turnover is considered to be correlated with transformation by some types of oncogenes and cellular response to growth factors such as epidermal growth factor (EGF)[74] and platelet-derived growth factor (PDGF).[75] Piericidin-B1 *N*-oxide (**18**) and B5 *N*-oxide (**19**) inhibited the EGF-induced PI turnover of A431 cells with an IC_{50} of 1.2 and 1.1 μg ml^{-1}, respectively, whereas piericidin B (**3**) and B5 (**20**) showed weaker inhibitory activity (IC_{50} 5.0 and 10.0 μg ml^{-1}, respectively) than the *N*-oxides (**18** and **19**).[19,76] *N*-oxide **18** showed no inhibitory effects on DNA, RNA or protein synthesis, at the concentration inhibiting PI.[77] Glucopiericidin A (**32**) inhibited PDGF-induced activation of phospholipase-C gamma 1 by reducing the tyrosine kinase activity of the PDGF receptor and it more potently inhibits PI turnover induced by PDGF than by EGF.[78]

The adenovirus E1A gene product inactivates retinoblastoma tumor suppressor protein (pRB), which has an important role in cell cycle and apoptosis control in mammalian cells, thereby stimulating host cell DNA synthesis.[79] Piericidins A, A2, C7, and C8 (**1**, **4**, **28** and **29**) showed selective cytotoxicities against rat glia cells transformed with the adenovirus E1A gene (RG-E1A-7) and inhibited the growth of Neuro-2a cells. Mitochondrial dysfunction might be one of the mechanisms causing cell death or growth arrest in certain types of tumors.[24]

Glucopiericidin A (**32**) was discovered as a filopodia protrusion inhibitor, but only synergistically with the mitochondrial respiration inhibitor piericidin A (**1**). It was shown that **32** suppresses glycolysis by functionally targeting the glucose transporter. Simultaneous inhibition of both glycolysis and mitochondrial respiration markedly decreased intracellular ATP levels, indicating that **32** inhibits ATP-dependent filopodia protrusion in carcinoma cells when treated with **1**.[80]

Glucose deprivation causes upregulation of GRP78 and induction of etoposide resistance in human cancer cells, thus downregulating GRP78 expression may be a novel strategy toward anticancer drug development. It was shown that piericidin A (**1**) suppresses the accumulation of GRP78 protein and was also highly toxic to etoposide-resistant HT-29 cells, with IC_{50} values for colony formation of 6.4 and 7.7 nM under 2-deoxyglucose supplemented and glucose-deprived conditions, respectively. Interestingly, **1** had no effect under normal growth conditions.[81]

JBIR-02 (**25**) was discovered as a regulator of arrestin translocation and was suggested to be a new type of antitumor drug. Compounds **25** and **1** inhibited nuclear export of β-arrestin 2-EYFP (enhanced yellow fluorescent protein) fusion protein in HeLa cells at concentrations of 20 and 40 μM, respectively, whereas IT-143-B (**24**) had no such effect.[21]

Animal experiments showed several piericidins could be promising as lead compounds for antitumor drug development. An i.p. injection, at 0.625 mg kg^{-1}, of 3′-deoxytalopiericidin-A1 (**39**) resulted in the suppression of the growth of colon 26 tumor (T/C = 21.8%) implanted s.c. in syngeneic CDF1 mice.[32] Piericidin B1 *N*-oxide (**18**) reversibly inhibited the growth of A431 cells *in situ* and suppressed the growth of Ehrlich carcinoma *in vivo* when administered to mice by i.p. injection.[77]

Other bioactivities

Studies of piericidin A (**1**) on the metabolism of isolated adipose cells showed the inhibition of glucose and fructose utilization, and the elimination of the stimulatory effects of proteases on glucose utilization. Piericidin A (**1**) accelerated glycogenolysis in isolated adipose cells, but exerted no significant effects on the level of lipid content or the oxidation of the cellular components. Lipolysis induced by lipolytic hormones or phosphodiesterase inhibitors, or by both, was also blocked by **1**. The effects of **1** on the isolated adipose cells were interpreted as probably being due to the plasma membrane effects and possible effects on the adenylyl (adenyl) cyclase system.[82]

The effects of **1**, glucopiericidins A (**32**) and B (**33**) on antibody formation to sheep red blood cells in mouse spleen cell cultures were examined. D-Glucose in the glucopiericidins seemed to be necessary for inhibitory activity, as **1** was less effective than the glucosylated analogs in tests.[28] Piericidin A (**1**) was identified as a selective inhibitor of phagocytosis,[83] one of the basic and characteristic properties of macrophages. In addition, **1** was also shown to inhibit the cytokine interleukin-2 production in mouse thymoma EL4 cells, which regulates many essential immune functions.[84]

Mer-A2026A and B (**21** and **22**) were found to have potent vasodilating activities.[76] The vasodilating effects of **21** and **22** on rat aorta were greater than that of papaverine, a standard vasodilating drug. Mer-A2026A (**21**; 0.1 mg kg^{-1}), as well as papaverine (1 mg kg^{-1}), showed similar potent depressor effects in spontaneously hypertensive rats, but the duration of the effect caused by **21** was significantly longer than that by papaverine.[72]

The type III secretion system (T3SS) is a bacterial appendage used by dozens of Gram-negative pathogens to subvert host defenses and cause disease, making it an ideal target for pathogen-specific antimicrobials.[85] Compounds **22** and **1** were shown to inhibit *Yersinia pseudotuberculosis* from triggering T3SS-dependent activation of the host transcription factor NF-κB in HEK293T cells, but were not toxic to mammalian cells at comparable concentrations. Compounds **22** and **1** were described as bacterial T3SS inhibitors less likely to generate resistance.[85]

CONCLUSIONS

The family of piericidin metabolites are some of the most commonly encountered in culture studies of the actinomycetes isolated from soil and marine samples. Forty piericidins, including nine piericidin glycosides, have been isolated to date. The common genus *Streptomyces* is the main source of the piericidins, with occassional exceptions of piericidin metabolites found produced by *Nocardioides* species. (Table 1). In those identified *Streptomyces* strains, *S. pactum* is a well-known piericidin producer. Although soil-derived actinomycetes have been the predominant source of the piericidins, more recently, marine-derived *Streptomyces* isolates have shown promise to produce piericidins, especially new piericidin analogs.[24,33]

Piericidins are well known as complex I inhibitors. Moreover, some piericidins, such as 3′-deoxytalopiericidin-A1(**39**)[32] and piericidin B1 *N*-oxide (**18**), are promising as lead compounds for anti-cancer drug development.[76,77] SAR studies indicate that the sugar component of the piericidin glycosides is important in modulating their physiological activities.[33] However, the bioactivities of the piericidin glycosides seem much less well defined and require further refinement, especially regarding their anticancer mechanism of action. Overall, the broad bioactivity of this class of microbial metabolites seems less well understood. Clearly many are potently toxic. Given the potency in a diversity of bioassay systems, the piericidins are likely to captivate interest for some time to come.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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