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New insights into the antitumor potential of natural piericidins

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[Abstract] Piericidins are a family of *α*-pyridone antibiotics with fascinating biological activity produced by actinomycetes, derived from land soil, insect or marine samples. There are 39 natural piericidins reported before 2016, and most of them are obtained from soil actinomycetes. However, marine-derived *Streptomyces* isolates have been showed great importance to produce piericidins with new structures in recent 5 years. This review covers the 40 natural piericidins reported after 2016, together with their obvious cytotoxic activities against cancer cell lines. Their structure–activity relationships are also discussed briefly. The anti-tumor potential, especially as lead compounds for anti-renal cell carcinoma agents, is of great concern recently. This review helps to provide comprehensive chemical information and new insights into the antitumor potential of piericidins.
[Key words] Natural piericidins; Antitumor potential; Structure-activity relationships

1 Introduction

Piericidins are a family of *α*-pyridone antibiotics, feature 4-pyridinol core linked with a methylated polyketide side chain, exclusively produced by actinomycetes, derived from land soil, insect, or marine samples. The close structural similarity with coenzyme Q renders the piericidins important NADH-ubiquinone oxidoreductase (Complex I) inhibitors in the mitochondrial electron transport chain, resulting in fascinating biological activity such as antimicrobial and insecticidal activities. Our previous review in 2016 provided a comprehensive overview of 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[1]. In recent 5 years, piericidins have attracted more attention. More natural piericidins with new structures have been discovered from actinomycetes strains, especially from the marine-derived *Streptomyces*. Their anti-tumor potential, especially as lead compounds for anti-renal cell carcinoma agents, has been well studied. Our group has made an important

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contribution in those studies. Therefore, in order to give the authors an updated and comprehensive understanding, this review summarizes the progress of the natural product research of piericidins and focused on the potential antitumor effects.

2 Natural Piericidins

Piericidin A (**1**, also named piericidin A1) was reported from the soil-derived actinomycete *Streptomyces mobaraensis*, as a new insecticidal metabolite[2]. As introduced in our previous review in great detail, there are 39 natural piericidins reported before 2016 (Fig.1)[1]. *Streptomyces* is the main source of those piericidins, except piericidins C6 (**26**) only from a *Nocardioides* sp., while piericidins C2 and C4 (**10** and **12**) both from a *Nocardioides* sp. and *Streptomyces pactum*[3]. Soil actinomycetes was the only resource of natural piericidins compounds in last century, however, marine-derived *Streptomyces* isolates showed great importance to produce piericidins with new structures, by the development of marine natural products and marine microorganism studies from 2000.

In recent years, our research group focused on exploring natural piericidins in marine-derived

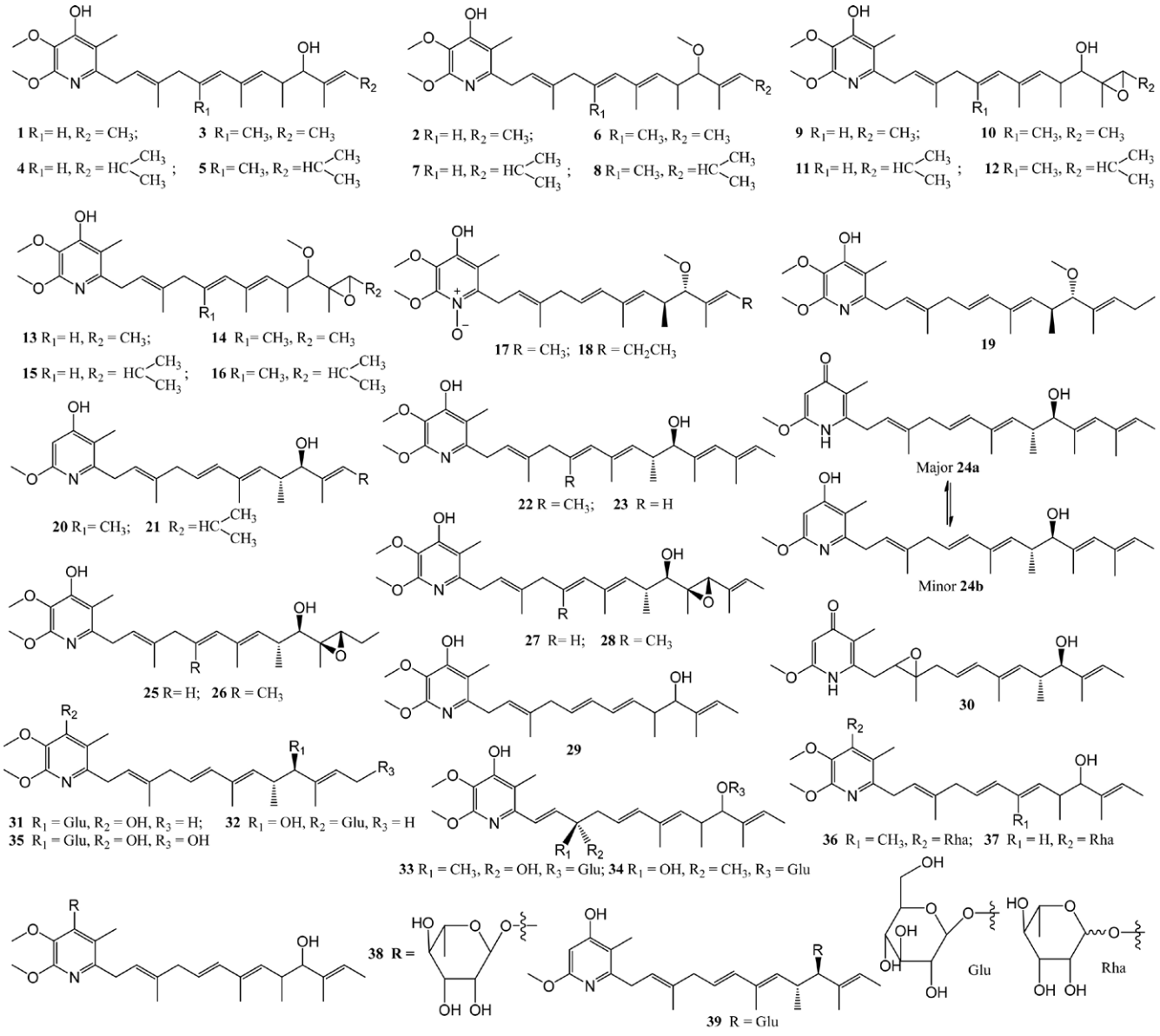

Fig. 1 Natural piericidins 1-39

Streptomyces strains. Chemical dereplication by high-performance liquid chromatography/mass spectrometry (HPLC/MS) was performed to screen the strains with piericidins in marine plants and animals-derived and marine sediment (including mangrove sediment) *Streptomyces* strains in our lab. Two actinomycete strains, *Streptomyces iakyrus* SCSIO NS104 and *Streptomyces psammoticus* SCSIO NS126, isolated from a mangrove sediment sample collected from the Pearl River estuary to South China Sea, have been uncovered with abundant and diverse piericidin metabolisms. Iakyricidins A-D (**40-43**) were isolated from the strain *Streptomyces iakyrus* SCSIO NS104[4]. Iakyricidin A (**40**, also named piericidin G in the patent), present the first natural carbonyl-containing piericidin derivate. Iakyricidins B-D (**41-43**), three novel piericidin analogues with a five membered ring moiety, were obtained and considered to be biosynthesized by uncommon C-C cyclization and double bond rearrangement in the polyene side chain[4]. In a culture extract of the strain *Streptomyces psammoticus* SCSIO NS126, 27 natural piericidins were isolated, including 4 new piericidin aglycones (**44-47**) and 13 new piericidin glycosides (**48-60**)[5]. Four aglycones were identified as piericidin H (**44**, 11-demethyl-piericidin A), piericidin I (**45**, 13*S*,19*R*-dihydroxyl-IT-143-A), piericidin J (**46**, 13*R*,19*S*-dihydroxyl-IT-143-A), and piericidin K (**47**, 10-ketone piericidin A). And those glycosides were identified as 7-demethylglucopiericidin A (**48**), 7-demethyl-13-hydroxyglucopiericidin A (**49**), 13-hydroxypiericidin A 10-*O*-*α*-D-galactose (1→6)-*β*-D-glucoside (**50**), 3-hydroxypiericidin A 10-*O*-*α*-D-glucose (1→6)-*β*-D-glucoside (**51**), 4'-*O*-*β*-D-glucose glucopiericidin A (**52**), 4'-*O*-*β*-D-glucose 13-hydroxyglucopiericidin A (**53**), 4'-*O*-*β*-D-glucose piericidin A 10-*O*-*α*-D-glucose (1→6)-*β*-D-glucoside (**54**), 5-hydroxy-6-hydroxymethyl glucopiericidin A (**55**), 5-hydroxy-6-hydroxymethyl-13-hydroxyglucopiericidin A (**56**), 2-hydroxymethyl-Δ3, 4-glucopiericidin A (**57**), 11*S*,12*R*-piericidin C1 10-*O*-*β*-*D*-glucoside (**58**), 11*R*,12*S*-piericidin C1 10-*O*-*β*-*D*-glucoside (**59**), and 13-dimethoxy glucopiericidin A (**60**) [5]. In order to explore more diverse piericidin derivatives, our group sought to optimize the fermentation conditions of the NS126 strain by adjusting the pH and choosing the culture time, as well as engaging in large-scale fermentation (300 L). In addition to the isolation and accumulation of the reported active piericidins, eight new piericidin natural products, piericidin L (**61**), piericidin N (**62**), piericidin Q (**63**), piericidin O (**64**), piericidin P (**65**), piericidin M (**66**), piericidin R (**67**) and 11-demethyl-glucopiericidin A (**68**), in trace amounts (about 1-3 mg) were obtained and identified (Fig.2)[6].

In addition, natural products study of the deletion mutant of reedsmycin biosynthetic genes from a reed rhizosphere soil derived *Streptomyces* sp. CHQ-64, led the discovery of piericidin F (**69**, 10-methoxyl Mer-A2026B)[7]. Glucopiericidinol A3 (**70**) and 7-demethyl-glucopiericidin A1 (**71**) were isolated from the culture broth of *Streptomyces* sp. KIB-H1083, an endophyte isolated from traditional Chinese medicinal plant *Diaphasiastrum veitchii*[8]. *Altogether* 25 new piericidin derivatives were reported from symbiont between beewolf and *Streptomyces* sp.[9], with their structures deduced by LCMS in situ analysis. Those molecules were not isolated as pure compounds and identified by NMR (nuclear magnetic resonance) and other spectroscopy. Therefore, the chemical structures of these derivatives could not be completely determined. The known piericidin derivatives, piericidin C and piericidin group antibiotic IT-143-B, were characterized by HPLC-HR-ESIMS (high-performance liquid chromatography-high resolution-electrospray ionization mass spectrometry) analysis from the culture broth of the marine sponge-associated *Nocardiopsis* sp. UR67[10]. The known piericidin derivatives Mer-

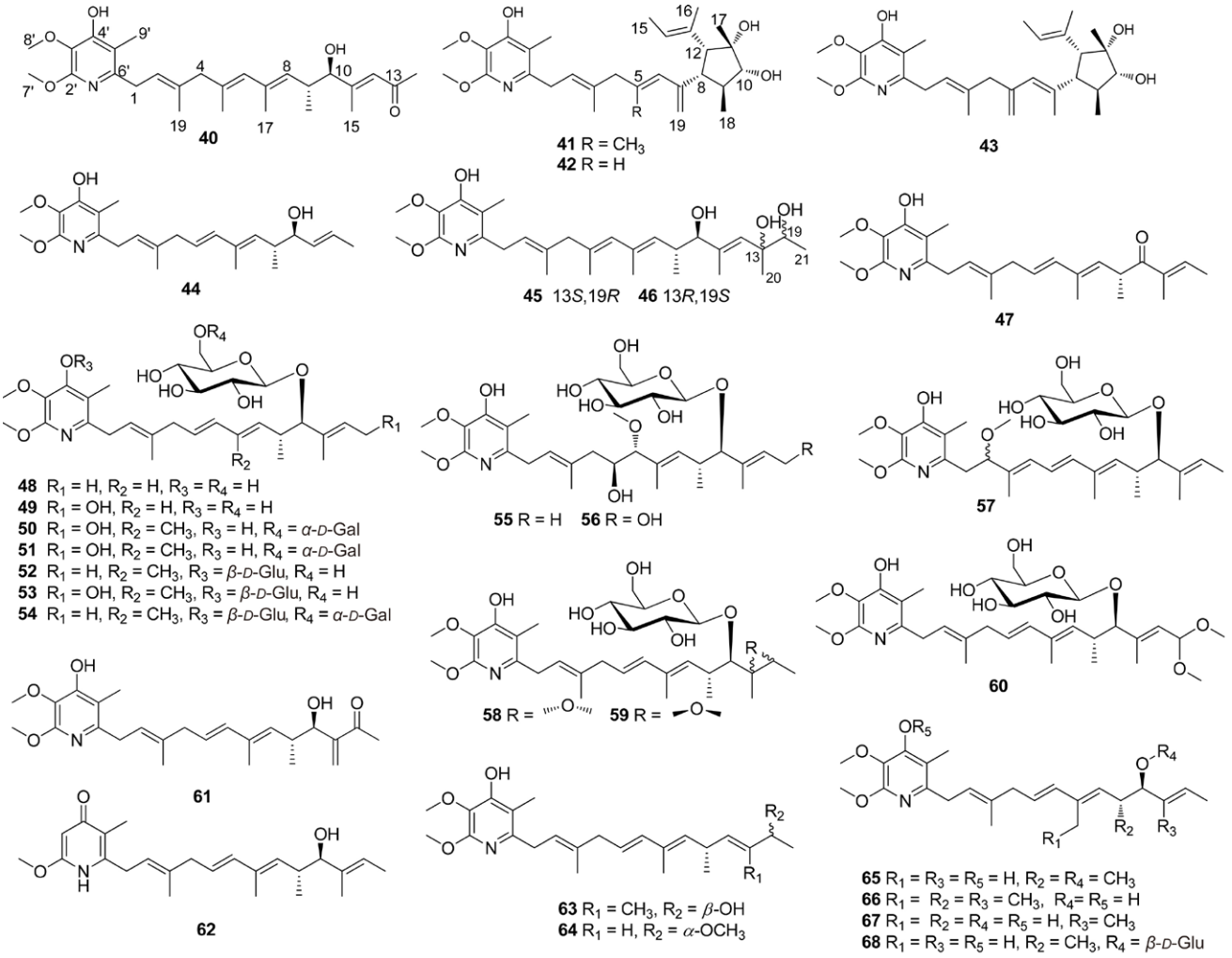

Fig. 2 Natural piericidins 40-68

A2026B and piericidin A1 were also isolated from cultures of *Streptomyces* sp. ICBG292, associated with attine ants[11]. Two new piericidins A5 (**72**) and G1 (**73**), a previously synthesized piericidin G2 (**74**), and two known piericidins A1 and A2 were isolated from the marine-derived *Streptomyces* sp. SCSIO 40063[12]. Five new piericidin glycosides (compounds 3, 4, 6, 7 and 8 in original paper) (**75-79**) were generated following the overexpression of glycosyltransferases-coding genes in a piericidin producer, marine-derived *Streptomyces youssoufiensis* OUC6819[13] (Fig.3).

3 Cytotoxic activities

Most of the natural piericidins showed cytotoxic activities against several cancer cell lines as listed in the Table 1. As discussed in the previous review, 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 significantly reduce the cytotoxic activity. 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. The biological activities of the piericidin glycosides are strongly influenced by the type and location of the sugar unit [1].

In the recent studies, most of the obtained piericidins were evaluated for their cytotoxicities against three renal carcinoma cell lines, ACHN, OS-RC-2, and 786-O. Most of the piericidins showed strong or moderate cytotoxicities toward ACHN, and several analogs showed significant

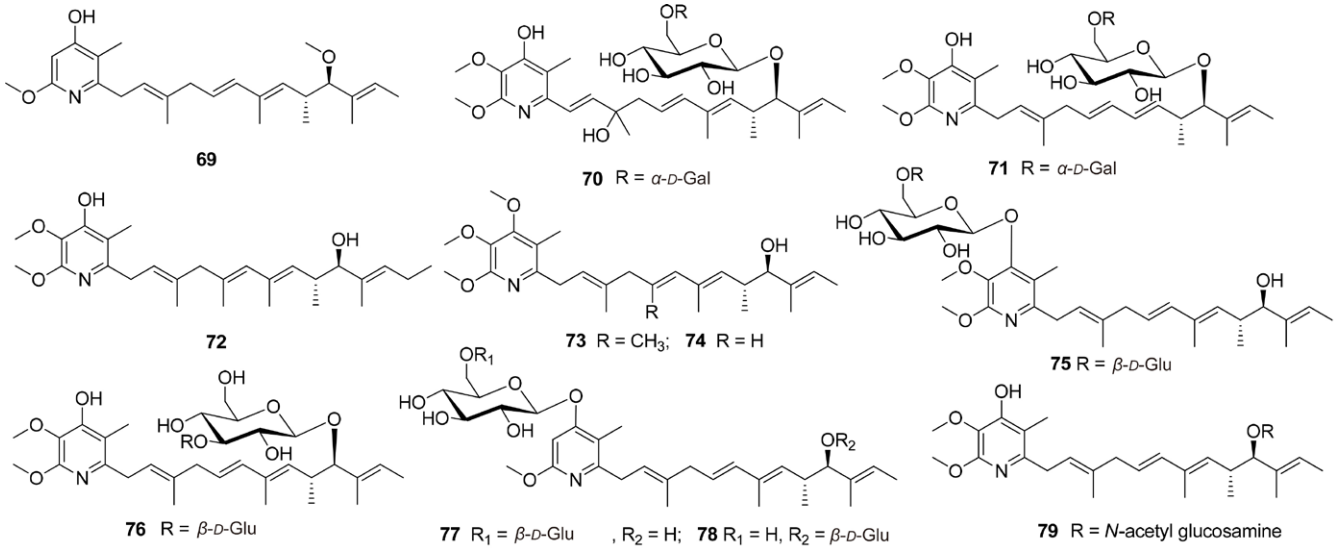

Fig. 3 Natural piericidins 69-79

Table 1 Cytotoxic activities of natural piericidins

Compounds	Cells (IC_{50})
Piericidin A (A1, **1**)	KB (8.9 μg/mL), K562 (>12.5 μg/mL), RG-E1A-7 (0.20 nM), Neuro-2a (0.21 nM), L1210 (5 nM)[1], ACHN (0.4 μM), OS-RC-2 (5.2 μM), 786-O (30 μM) [5], HL-60 (8.5 μM), K562 (2.4 μM), MOLT-4 (25 μM)[6]
Piericidin B (B1, **3**)	L1210 (6 nM)[1]
Piericidin A2 (**4**)	RG-E1A-7 (0.47 nM), Neuro-2a (0.22 nM)[1], ACHN (14 μM), OS-RC-2 (17 μM), 786-O (50 μM)[5]
Piericidin C2 (**11**)	ACHN (>100 μM), OS-RC-2 (22 μM), 786-O (>100 μM)[5]
IT-143-A (**23**)	ACHN (22 μM), 786-O (61 μM), OS-RC-2 (21 μM)[4]
IT-143-B (**24**)	ACHN (98 μM), 786-O (>100 μM), OS-RC-2 (22 μM)[4]
Piericidin C7 (**28**)	RG-E1A-7 (1.5 nM), Neuro-2a (0.83 nM)[1]
Piericidin C8 (**29**)	RG-E1A-7 (0.45 nM), Neuro-2a (0.21 nM)[1]
7-Demethylpiericidin A1 (**30**)	KB (11.0 μg/mL), K562 (>12.5 μg/mL)[1]
Glucopiericidin A (**32**)	HeLa S3 (0.25, 0.11 μg/mL), B16 (0.007 4 μg/mL), H-69 (0.019 μg/mL), P388 (0.36 μg/mL), P388/ADM (0.25 μg/mL)[1], ACHN (0.21 μM), OS-RC-2 (>100 μM), 786-O (>100 μM)[5], HL-60 (0.34 μM), SMMC-772 (0.65 μM), A-549 (0.60 μM), MCF-7 (0.50 μM)[8], A549(2.42 μM), A375(0.8 μM), HCT-116(0.16 μM), HT-29(0.97 μM)[13]
Glucopiericidinol A1 (**34**)	HeLa S3 (0.39, 0.62 μg/mL), B16 (0.32 μg/mL), H-69 (0.47 μg/mL), P388 (0.58 μg/mL), P388/ADM (4.3 μg/mL)[1], HL-60 (0.28 μM), SMMC-772 (5.32 μM), A-549 (9.92 μM), MCF-7 (2.69 μM)[8]
Glucopiericidinol A2 (**35**)	HeLa S3 (0.10, 0.98 μg/mL), B16 (0.67 μg/mL), H-69 (0.83 μg/mL), P388 (1.6 μg/mL), P388/ADM (4.2 μg/mL)[1]
13-Hydroxyglucopiericidin A (**36**)	HeLa S3 (0.76 μg/mL), B16 (0.21 μg/mL), H-69 (0.066 μg/mL), P388 (2.5 μg/mL), P388/ADM (0.78 μg/mL)[1], ACHN (7.21 μM), OS-RC-2 (>100 μM), 786-O (>100 μM)[5]
3'-Rhamnopiericidin A1 (**37**)	HeLa (2.8 μg/mL), KB (0.74, 3.8 μg/mL), K562 (4.0 μg/mL)[1]

Table 1 (Continued)

Compounds	Cells (IC_{50})
7-Demethyl-3'-rhamnopiericidin A1 (**38**)	KB (4.5 μg/mL), K562 (2.9 μg/mL)[1]
3'-Deoxytalopiericidin A1 (**39**)	Colon 26 (0.81 μg/mL), L1210 (7.91 μg/mL)[1]
Glucopiericidin C (**40**)	36 human tumor cell lines (mean 2.0 μM)[1], ACHN (3.8 μM), OS-RC-2 (14 μM) ,786-O (15 μM)[5]
Iakyricidin A (**40**, piericidin G)	ACHN (0.02 μM), 786-O (89 μM), OS-RC-2 (30 μM)[4]
Iakyricidin B (**41**)	ACHN (13 μM), 786-O (31 μM), OS-RC-2 (13 μM)[4]
Iakyricidin C (**42**)	ACHN (69 μM), 786-O (84 μM), OS-RC-2 (21 μM)[4]
Iakyricidin D (**43**)	ACHN (42 μM), 786-O (62 μM), OS-RC-2 (31 μM)[4]
Piericidin H (**44**)	ACHN (4.1 μM), OS-RC-2 (11 μM), 786-O (>100 μM)[5]
Piericidin I (**45**)	ACHN (2.4 μM), OS-RC-2 (5.3 μM), 786-O (>100 μM)[5]
Piericidin J (**46**)	ACHN (3.8 μM), OS-RC-2 (4.1 μM), 786-O (>100 μM)[5]
Piericidin K (**47**)	ACHN (>100 μM), OS-RC-2 (22 μM), 786-O (>100 μM)[5]
7-Demethylglucopiericidin A (**48**)	ACHN (0.31 μM), OS-RC-2 (2.6 μM), 786-O (0.99 μM)[5]
7-Demethyl-13-hydroxyglucopiericidin A (**49**)	ACHN (2.5 μM), OS-RC-2 (79 μM), 786-O (28 μM)[5]
13-Hydroxypiericidin A 10-*O*-*α*-D-galactose (1→6)-*β*-D-glucoside (**50**)	ACHN (>100 μM), OS-RC-2 (>100 μM), 786-O (>100 μM)[5]
3-Hydroxypiericidin A 10-*O*-*α*-D-glucose (1→6)-*β*-D-glucoside (**51**)	ACHN (23 μM), OS-RC-2 (>100 μM), 786-O (>100 μM)[5]
4'-*O*-*β*-D-Glucose glucopiericidin A (**52**)	ACHN (28 μM), OS-RC-2 (>100 μM), 786-O (>100 μM)[5], A549 (1.12 μM), A375 (0.74 μM), HCT-116 (0.27 μM), HT-29 (2.34 μM)[13]
5-Hydroxy-6-hydroxymethyl glucopiericidin A (**55**)	ACHN (2.4 μM), OS-RC-2 (>100 μM), 786-O (>100 μM)[5]
5-Hydroxy-6-hydroxymethyl-13-hydroxyglucopiericidin A (**56**)	ACHN (60 μM), OS-RC-2 (>100 μM), 786-O (>100 μM)[5]
2-Hydroxymethyl-Δ3, 4-glucopiericidin A (**57**)	ACHN (1.7 μM), OS-RC-2 (60 μM), 786-O (28 μM) [5]
11*S*,12*R*-Piericidin C1 10-*O*-*β*-D-glucoside (**58**)	ACHN (2.8 μM), OS-RC-2 (30 μM), 786-O (19 μM) [5]
Piericidin L (**61**)	ACHN (>50 μM), 786-O (>50 μM), OS-RC-2 (2.2 μM), HL-60 (12 μM), K562 (>50 μM), MOLT-4 (>50 μM)[6]
Piericidin N (**62**)	ACHN (>50 μM), 786-O (>50 μM), OS-RC-2 (42.9 μM), HL-60 (0.08 μM), K562 (>50 μM)[6]
Piericidin Q (**63**)	ACHN (>50 μM), 786-O (>50 μM), OS-RC-2 (>50 μM), HL-60 (0.08 μM), K562 (>50 μM)[6]

Table 1 (Continued)

Compounds	Cells (IC_{50})
Piericidin O (**64**)	ACHN (>50 μM), 786-O (>50 μM), OS-RC-2 (>50 μM), HL-60 (0.08 μM), K562 (>50 μM)[6]
Piericidin P (**65**)	ACHN (>50 μM), 786-O (>50 μM), OS-RC-2 (>50 μM), HL-60 (0.1 μM), K562 (>50 μM)[6]
Piericidin M (**66**)	ACHN (>50 μM), 786-O (>50 μM), OS-RC-2 (4.5 μM), HL-60 (9.8 μM), K562 (>50 μM), MOLT-4 (>50 μM)[6]
Piericidin R (**67**)	ACHN (>50 μM), 786-O (>50 μM), OS-RC-2 (15 μM), HL-60 (>50 μM), K562 (>50 μM), MOLT-4 (>50 μM)[6]
11-Demethyl-glucopiericidin A (**68**)	ACHN (2.3 μM), 786-O (12.0 μM), OS-RC-2 (28.7 μM), HL-60 (1.3 μM), K562 (5.5 μM)[6]
Piericidin F (**69**)	HeLa (0.003 μM), NB4 (0.037 μM), A549 (0.56 μM), H1975 (0.49 μM)[7]
Glucopiericidinol A3 (**70**)	HL-60 (>40 μM), SMMC-772 (>40 μM), A-549 (>40 μM), MCF-7 (>40 μM), SW480 (>40 μM)[8]
7-Demethyl-glucopiericidin A1 (**71**)	HL-60 (>40 μM), SMMC-772 (21.66 μM), A-549 (22.78 μM), MCF-7 (10.88 μM), SW480 (>40 μM)[8]
Piericidin G1(**73**)	SF-268, MCF-7, HepG2 and A549 with IC_{50} values between 10.0 and 12.7 μM[12]
75	A549 (9.57 μM), A375 (4.3 μM), HCT-116 (2.07 μM), HT-29 (5.94 μM)[13]
77	A549 (24.8 μM), A375 (>25 μM), HCT-116 (5.03 μM), HT-29 (13.93 μM)[13]
78	A549 (19.45 μM), A375 (2.73 μM), HCT-116 (2.55 μM), HT-29 (6.45 μM)[13]
79	A549 (15.72 μM), A375 (3.11 μM), HCT-116 (1.47 μM), HT-29 (7.43 μM)[13]

activities with IC_{50} values less than 1 μM (Table 1). OS-RC-2, 786-O, and HK-2 cells were not as sensitive as ACHN to most of the piericidins[5]. Iakyricidin A (**40**, piericidin G) showed the strongest and selective antiproliferative activities toward ACHN cells with an IC_{50} value of 20 nM, and it is suggested that the α, β-unsaturated ketone group in iakyricidin A as a Michael acceptor might improve the inhibitory activity[4]. Another carbonyl-containing piericidin L (**61**) and piericidins M (**66**) were revealed with selective cytotoxic activities against OS-RC-2 cells[6]. Piericidin F was also attractive with stronge cytotoxicity against the HeLa, NB4, A549, and H1975 cell lines with IC_{50} values of 0.003, 0.037, 0.56, and 0.49 μM[7].

According to the cytotoxicity data of these natural piericidin analogues, especially those against several renal carcinoma cell lines, structure-activity relationships (SAR) are discussed briefly. Although the previously SAR studies indicate that the sugar component of the piericidin glycosides is important in modulating their physiological activities[1], there was no definitive evidence about that who (piericidin aglycones or glycosides) had greater antitumor activity. However, piericidin glycosides appear to be more selective for renal carcinoma cell lines[5]. Analogs with the methoxy group lost at C-3′ of pyridone or hydroxypyridine moiety has not shown the significant activity against renal cancer cells, but show prominent potential against other tumor cells, like **62** against HL-60, and **69** against HeLa, NB4, A549, and H1975 cells. More oxidation, hydroxylation or cyclization on the branched chain reduce the cytotoxicity and selectivity of the piericidins, but it is suggested that a carbonylated branched chain could greatly enhance the activities, such as **40** and **61**[6]. These SARs are of significance for further

structural modification and optimization of lead compounds, as well as drug development.

4 Mechanism of anti-renal cell cancer and toxicology

By exploring the mechanisms of two representative natural piericidin compounds, piericidin A (PA) and glucopiericidin A (GPA), peroxiredoxin 1 (PRDX1) is detected as a potential target by transcriptome data of PA treated ACHN cells, as well as the paired RCC (renal cell carcer) tumour *vs.* adjacent non-tumour tissues. Remarkably, piericidins (PA/GPA) target PRDX1 (peroxiredoxin 1), increasing the mRNA and protein levels of PRDX1, which reduce ROS (reactive oxygen species) levels in ACHN cells along with obvious cell apoptosis induction and proliferation inhibition. Besides, PA/GPA can interact directly with PRDX1 protein and force it into nucleus, thereby inhibiting the activation of key genes in the renal caner pathway. Moreover, our study revealed that ACHN cells were less sensitive to piericidins when PRDX1 was downregulated. Therefore, our direct evidence shows that PA/GPA targets PRDX1 and reduces ROS in ACHN cells. PA and GPA were investigated with their molecular mechanisms on renal carcinoma cells, as well as the *in vivo* anti-cancer evaluation. PA and GPA were being chosen as representative molecules of piericidin aglycones and glycosides, respectively, because of their relative high yields in the *Streptomyces* strain and their significant inhibitory ability against ACHN cells *in vitro*. There were no obvious differences between the piericidin glycosides and aglycones in the cytotoxicity against ACHN cells. Moreover, the nearly indistinguishable anti-cancer effects *in vivo* between PA and GPA in our study suggest that the same metabolic substances might contribute to the efficacy after drug metabolism *in vivo*. However, the upregulation of PRDX1 protein expression by GPA in the nucleus of ACHN cells appears stronger than that of PA. And the kinetic constant in surface plasmon resonance (SPR) test also showed glycoside GPA bound more effectively than the aglycone PA with PRDX1. In this study, we found that PRDX1 could be used as an important new target for anti-RCC agents[5].

In nude mice bearing ACHN xenografts, PA and GPA exhibit hepatotoxicity. Proteomics and transcriptomics reveal that the hepatotoxicity related with cholesterol disposition since RCC is characterized by cholesterol accumulation. PA/GPA aggravate hepatotoxicity in HCD (high cholesterol diet)-fed mice while exhibit no toxicity in chow diet-fed mice. High cholesterol accumulation in liver is LXR (liver X receptor)-mediated CYP7A1 (cholesterol 7α-hydroxylase) depression and LDLR (low density lipoprotein receptor) activation. FXR (farnesoid X receptor) is also depressed with downregulated target gene OSTα. Different from PA directly combined with LXRα as an inhibitor, GPA exists as a prodrug in liver and exerts toxic effect due to transform into PA. SPR and docking results of 17 piericidins illustrate glycosides exert no LXRα binding activity. Longer survival time of GPA-treated mice indicates further exploration in anti-RCC drug research should focus on reducing the transformation of glycosides into PA and concentrating them in kidney tumor rather than liver for lowering hepatotoxicity risk[14]. In PK study, a small beneficial effect of GPA on survival time was also observed, in which mice was alive after GPA treatment but dead after PA administration in the same dose within 45 minutes. Accordingly, PA was reported as the inhibitor of respiratory complex I, which induced respiratory depression or hypoxic pulmonary vasoconstriction (HPV)[15], might take account for the shorter survival time of PA.

5 Perspective

In recent years, natural products study and antitumor effects of piericidins show that those actinomycetes-produced antibiotics possess great potential in the development of antitumor drugs,

especially for renal cancer. As we known, the origin problem is inevitable for the natural-derived lead compounds in the process of drug development. Although it has been reported that marine microbial sources are abundant, they are not sufficient for preclinical studies. For the first isolation piericidin A, a complex total chemical synthesis of it has been achieved successfully[16]. In addition, a concise and convergent total synthesis with more efficient green approaches of piericidin A was reported in 2018[17]. However, the chemical synthesis of glycoside has not been reported. The research on biosynthesis and heterologous expression also provides ideas for the origin problem[13,18-19]. Of great concern, the recent researches of expression efficiency and biosynthetic regulatory mechanism provides insight into future molecular synthetic engineering construction of piericidins in response to pharmaceutical development[20-22], and might be one of the advantages of microbial-derived drug development.

Abbreviation

Complex I: NADH-ubiquinone oxidoreductase

HPLC/MS: High-performance liquid chromatography/mass spectrometry

PA: Piericidin A

GPA: Glucopiericidin A

PRDX1: Peroxiredoxin 1

RCC: Renal cell carcer

PRDX1: Peroxiredoxin 1

ROS: Reactive oxygen species

SPR: Surface plasmon resonance

HCD: High cholesterol diet

LXR: Liver X receptor

CYP7A1: Cholesterol 7α-hydroxylase

LDLR: Low density lipoprotein receptor

FXR: Farnesoid X receptor

HPV: Hypoxic pulmonary vasoconstriction

References

[1] Zhou XF, Fenical W. The unique chemistry and biology of the piericidins[J]. *J Antibiot*, 2016, 69(8):582-593.

[2] Tamura S, Takahashi S, Miyamoto S, et al. Isolation and physiological activities of piericidin A, a natural insecticide produced by *Streptomyces*[J]. *Agric Biol Chem*, 1963, 27(8):576-582.

[3] Kubota NK, Ohta E, Ohta S, et al. Piericidins C5 and C6:new 4-pyridinol compounds produced by *Streptomyces* sp. and *Nocardioides* sp.[J]. *Bioorg Med Chem*, 2003, 11(21):4569-4575.

[4] Li KL, Liang Z, Chen WH, et al. Iakyricidins A-D, antiproliferative piericidin analogues bearing a carbonyl group or cyclic skeleton from *Streptomyces iakyrus* SCSIO NS104[J]. *J Org Chem*, 2019, 84(19):12626-12631.

[5] Zhou XF, Liang Z, Li KL, et al. Exploring the natural piericidins as anti-renal cell carcinoma agents targeting peroxiredoxin 1[J]. *J Med Chem*, 2019, 62(15):7058-7069.

[6] Li KL, Su ZQ, Gao YL, et al. Cytotoxic minor piericidin derivatives from the actinomycete strain *Streptomyces psammoticus* SCSIO NS126[J]. *Mar Drugs*, 2021, 19(8):428.

[7] Han X, Liu Z, Zhang Z, et al. Geranylpyrrol A and piericidin F from *Streptomyces* sp. CHQ-64 Δ*rdmF*[J]. *J Nat Prod*, 2017, 80(5):1684-1687.

[8] Shang NN, Zhang ZX, Huang JP, et al. Glycosylated piericidins from an endophytic *Streptomyces* with cytotoxicity and antimicrobial activity[J]. *J Antibiot*, 2018, 71(7):672-676.

[9] Engl T, Kroiss J, Kai M, et al. Evolutionary stability of antibiotic protection in a defensive symbiosis[J]. *Proc Natl Acad Sci USA*, 2018, 115(9):E2020-E2029.

[10] Ibrahim AH, Attia EZ, Hajjar D, et al. New cytotoxic cyclic peptide from the marine sponge-associated *Nocardiopsis* sp. UR67[J]. *Mar Drugs*, 2018, 6(9):290.

[11] Ortega HE, Ferreira LG, Melo WGP, et al. Antifungal compounds from *Streptomyces* associated with attine ants also inhibit *Leishmania donovani*[J]. *PLoS Negl Trop Dis*, 2019, 13(8):e0007643.

[12] Peng J, Zhang QB, Jiang XD, et al. New piericidin derivatives from the marine derived *streptomyces* sp. SCSIO 40063 with cytotoxic activity[J]. *Nat Prod Res*, 2021, 19:1-7.

[13] Liu ZZ, Xiao F, Cai SQ, et al. Effective generation of glucosylpiericidins with selective cytotoxicities and insights into their biosynthesis[J]. *Appl Environ Microbiol*, 2021, 87(13):e00294-21.

[14] Liang Z, Chen YL, Gu TW, et al. LXR-mediated regulation of marine-derived piericidins aggravates high cholesterol diet-induced cholesterol metabolism disorder in mice[J]. *J Med Chem*, 2021, 64(14):9943-9959

[15] Dunham-Snary KJ, Wu DC, Potus F, et al. Ndufs2, a core subunit of mitochondrial complex I, is essential for acute oxygen-sensing and hypoxic pulmonary vasoconstriction[J]. *Circ Res*, 2019, 124(12):1727-1746.

[16] Lipshutz BH, Amorelli B. Total synthesis of piericidin A1. Application of a modified negishi carboalumination-nickel-catalyzed cross-coupling[J]. *J Am Chem Soc*, 2009, 131(4):1396-1397.

[17] Trost BM, Gholami H. Propene as an atom-economical linchpin for concise total synthesis of polyenes:piericidin A[J]. *J Am Chem Soc*, 2018, 140(37):11623-11626.

[18] Liu Q, Yao F, Chooi YH, et al. Elucidation of piericidin A1 biosynthetic locus revealed a thioesterase-dependent mechanism of alpha-pyridone ring formation[J]. *Chem Biol*, 2012, 19(2):243-253.

[19] Chen Y, Zhang W, Zhu Y, et al. Elucidating hydroxylation and methylation steps tailoring piericidin A1 biosynthesis[J]. *Org Lett*, 2014, 16(3):736-739.

[20] Peng QY, Gao GX, Lv J, et al. Engineered *Streptomyces lividans* strains for optimal identification and expression of cryptic biosynthetic gene clusters[J]. *Front Microbiol*, 2018, 9:3042.

[21] Li Y, Kong LX, Shen JF, et al. Characterization of the positive SARP family regulator PieR for improving piericidin A1 production in *Streptomyces piomogeues* var. Hangzhouwanensis[J]. *Synth Syst Biotechnol*, 2019, 4(1):16-24.

[22] Shen JF, Kong LX, Li Y, et al. A LuxR family transcriptional regulator AniF promotes the production of anisomycin and its derivatives in *Streptomyces hygrospinosus* var. beijingensis[J]. *Synth Syst Biotechnol*, 2019, 4(1):40-48.