Filamentous Fungi-Derived Orsellinic Acid-Sesquiterpene Meroterpenoids: Fungal Sources, Chemical Structures, Bioactivities, and Biosynthesis

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ABSTRACT

Fungi-derived polyketide-terpenoid hybrids are important meroterpenoid natural products that possess diverse structure scaffolds with a broad spectrum of bioactivities. Herein, we focus on an ever-increasing group of meroterpenoids, orsellinic acid-sesquiterpene hybrids comprised of biosynthetic start unit orsellinic acid coupling to a farnesyl group or/and its modified cyclic products. The review entails the search of China National Knowledge Infrastructure (CNKI), Web of Science, Science Direct, Google Scholar, and PubMed databases up to June 2022. The key terms include "orsellinic acid", "sesquiterpene", "ascochlorin", "ascofuranone", and "*Ascochyta viciae*", which are combined with the structures of "ascochlorin" and "ascofuranone" drawn by the Reaxys and Scifinder databases. In our search, these orsellinic acid-sesquiterpene hybrids are mainly produced by filamentous fungi. Ascochlorin was the first compound reported in 1968 and isolated from filamentous fungus *Ascochyta viciae* (synonym: *Acremonium egyptiacum; Acremonium sclerotigenum*); to date, 71 molecules are discovered from various filamentous fungi inhabiting in a variety of ecological niches. As typical representatives of the hybrid molecules, the biosynthetic pathway of ascofuranone and ascochlorin are discussed. The group of meroterpenoid hybrids exhibits a broad arrange of bioactivities, as highlighted by targeting hDHODH (human dihydroorotate dehydrogenase) inhibition, antitrypanosomal, and antimicrobial activities. This review summarizes the findings related to the structures, fungal sources, bioactivities, and their biosynthesis from 1968 to June 2022.

Introduction

Polyketide-terpenoid hybrids are well-known to be meroterpenoid natural products. They are widely distributed in the fungal kingdom, which displays potent activities and remarkable structures, exemplified by mycophenolic acids, yanuthones, and asperni-dines, as well as those that are derived from 3,5-demethylorsellinic acid, etc. [1–3].

Orsellinic acid-sesquiterpene meroterpenoids are a small group of polyketide-terpenoids. The hybrids are featured by the composition of orsellinic acid moiety and farnesyl group or/and its folded products with diverse scaffolds mainly transformed by terpene synthases. Without exception, the farnesyl part of all hybrids is attached to orsellinic acid moiety at the C-3 position (red bond). Mainly filamentous fungi are dominant producers that can inhabit a variety of ecological niches.

# Contributed equally

Due to their unique structures, as well as promising bioactivities, the small group of meroterpenoids has attracted considerable attention from chemists and biologists. Ascochlorin was the first example isolated from filamentous fungus *Ascochyta viciae* (*A. viciae*) in 1968, which has been later identified as *Acremonium egyptiacum* (synonym: *Acremonium sclerotigenum*) [4, 5]. Structurally, the structures of this group are reminiscent of vitamin E (γ-tocotrienol), the essential substance of mammalian health, and coenzyme Q10, as well as ubiquinone derivatives [6, 7]. Bedside-formed farnesyl variants, including monocyclic and bicyclic products, mediated by terpene cyclase and tailoring enzymes are responsible for further enlarging the chemical space. The sesquiterpene moiety is mainly decorated with etherification, acylation, oxidation, addition, rearrangement reaction, etc. Particularly, rearrangement reactions result in a change in carbon frameworks. In addition, various enzyme catalysts have been formed in nature to act on halogens in the skeleton through oxidation, reduction, and other strategies, playing an important role in the structural diversity and functional enrichment of natural products. Halogenation is a common modification reaction and usually occurs in biosynthetic start unit orsellinic acid at C-5. This halogenation process is catalyzed by the halogenase AscD, which performs or does not perform its function, resulting in different products obtained, such as compound **12** with chlorine and compound **13** without chlorine.

The diverse chemical space confers a broad range of biological activities, including antivirus, antitumor, anti-inflammatory, hypolipidemic, and anti-trypanosome. Because of structural similarity with ubiquinone derivatives, such as decylubiquinone, the group of meroterpenoids is highlighted by hDHODH (human dihydroorotate dehydrogenase) inhibiting activity. hDHODH is a key enzyme involved in the *de novo* pyrimidine biosynthesis, which is frequently overexpressed to support their growth of cancer cells. Inhibition of hDHODH activity has been proven to validate to suppress proliferation of cancer cells and represents a promising target for chemotherapeutic drugs [7–9]. The literature search strategy involved the search of the Web of Science, Science Direct, Google Scholar, PubMed, Reaxys, and Scifinder databases up to June 2022. The significant bioactivities of some compounds such as ascofuranone and ascochlorin have been intensively studied, and the reviews relating to bioactivities have been summarized [7, 10, 11].

In this review, we focus on the structure isolation, purification, filamentous fungi sources, and biological activities of orsellinic acid-sesquiterpene hybrids from 1968 to June 2022 (**Table 1S**, Supporting Information).

Overview

Orsellinic acid-sesquiterpene hybrids are almost produced by filamentous fungi. These producing fungi can widely inhabit various ecological niches, such as marine sponge, coral, terrestrial soil, etc. The original study of the hybrids can be traced back to 1968, and ascochlorin represent the first compound isolated from filamentous fungus *A. viciae*. This review summarizes that 71 orsellinic acid-sesquiterpene hybrids (**1–71**) have been isolated and identified from filamentous fungi over the past half century (until June 2022).

Based on the biosynthesis of the hybrids, these compounds are classified into three groups based on cyclization of the sesquiterpenoid part, namely, linear type, monocyclic type, and bicyclic type.

Biosynthesis

Since 1968, orsellinic acid-sesquiterpene hybrids were continually isolated; however, its biosynthetic pathway was not completely analyzed until 2019.

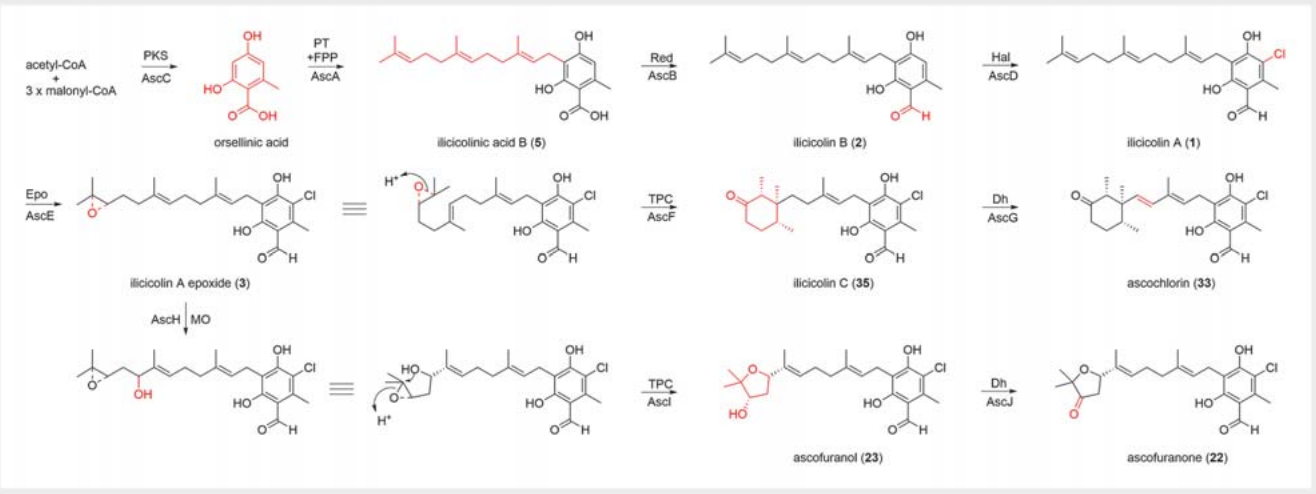
In 2016, the biosynthetic gene cluster for LL-Z1272β (2) from *Stachybotrys bisbyi* PYH05–7 was identified by Li et al. The heterologous expression in *Aspergillus oryzae* NSAR1 unveiled the basic biosynthetic route of LL-Z1272β, supported by the production of orsellinic acid and ilicicolinic acid B (5, also named as grifolic acid) [12].

Araki et al. reported the detailed biosynthetic pathway of ascofuranone (22) and ascochlorin (33) in *A. egyptiacum* in 2019 (▶ **Fig. 1**) [13]. Briefly, it turns out that orsellinic acid is synthesized from PKS (polyketide synthase) and serves as the starting unit of the hybrids. Then, the prenyltransferase AscA attaches a farnesyl diphosphate (FPP) group to the orsellinic acid skeleton at the C-3 position to form a linear meroterpenoid product, ilicicolinic acid B (5). For this class of orsellinic acid-sesquiterpene hybrids, post-biosynthetic decoration included chlorination, etherification, acylation, oxidation, and glycosylation, which increased structural and functional diversity of the polyketide-terpenoid hybrids. The post-modifications also sometimes played ecological roles, such as plant defense and inhibition of pathogen growth. The *Acremonium* sp. LG0808, a producer of polyketide-terpenoid hybrids, is a plant endophytic fungus that may interact between plants and microbes, thereby playing a role in plant defense. The carboxylic acid group of 5 was reduced to the aldehyde group by AscB to produce ilicicolin B (2). Subsequent chlorination occurs at C-5 under the catalysis of AscD and resulted in the production of ilicicolin A (1). AscE is responsible for the epoxidation reaction between C-10′ and C-11′, thereby forming the common intermediate LL-Z 1272α epoxide (3). Compound 3 is the common precursor, as the branching point of the biosynthetic pathways of 22 and 33. 3 was first modified by terpene cyclase AscF to generate monocyclic six-membered ring product ilicicolin C (35), and then oxidized by AscG to give 33. In ascofuranone biosynthesis branches, compound 3 comes through the hydroxylation at C-8′ by P450 monooxygenase to generate 23, followed by cyclization by AscF to finally produce 22 [7].

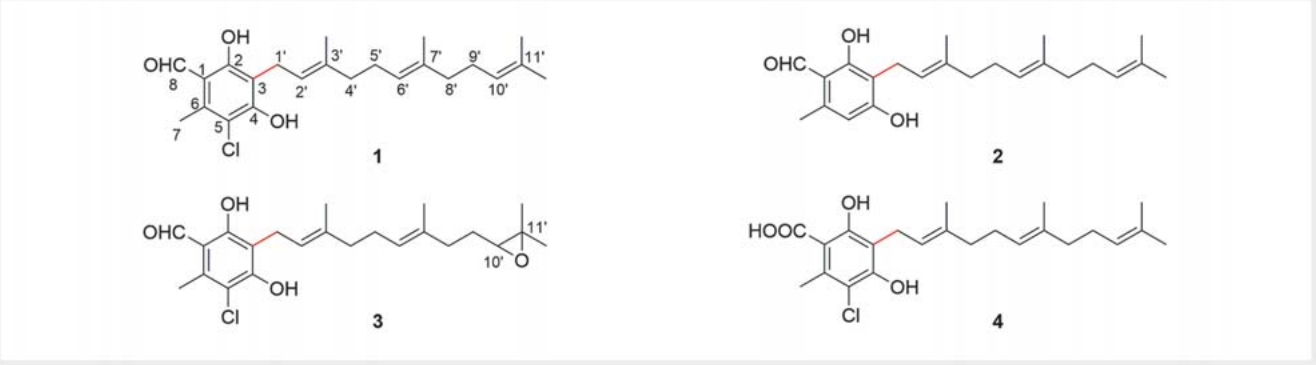
Linear Type

The linear type contains 21 molecules (**1–21**) in which the farnesyl group is formed to the linear sesquiterpene attached to orsellinic acid at C-3. Compounds **1** and **2** are the first reported cases belonging to this type. The linear ones are always co-isolated with other structure types and are usually considered as the biosynthetic precursors.

LL-Z 1272α (**1**) and LL-Z 1272β (**2**) (▶ **Fig. 2**), as anti-*Tetrahymena pyriformis* compounds, were originally isolated from filamentous fungi *Fusarium* sp. LL-Z 1272 in 1969. At the same time, or-



▶ **Fig. 1** The biosynthesis of ascofuranone (**22**) and ascochlorin (**33**). Enzymes are abbreviated as follows: PKS, polyketide synthase; PT, prenyltransferase; Red, reductase; Hal, halogenase; TPC, terpene cyclase; Dh, dehydrogenase; MO, monooxygenase; Epo, epoxidase [13].



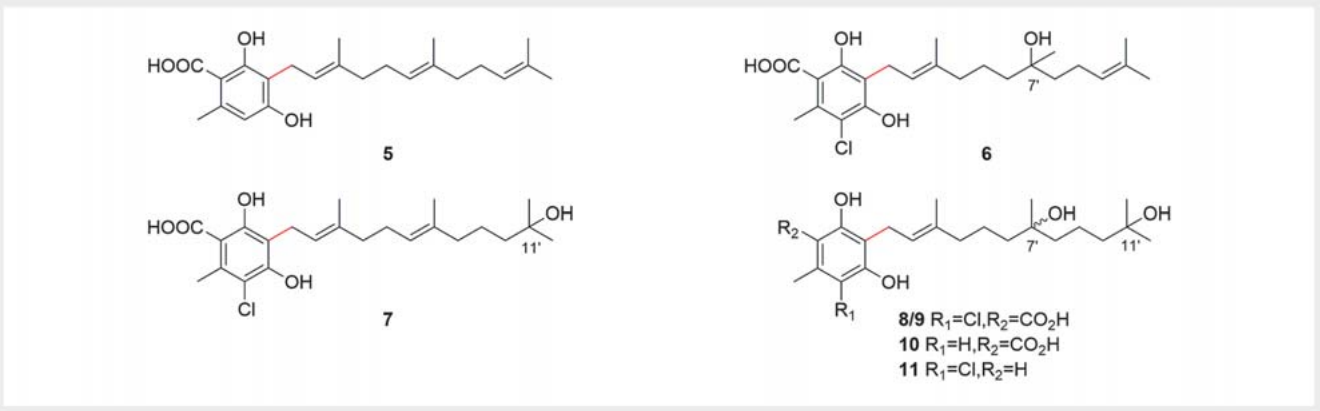
▶ **Fig. 2** Structures of compounds **1–4**. The red bond is to easily draw a distinction between PKS unit and terpene unit and to highlight the coupling position of two precursor units (the same below).

sellinic acid was co-isolated from fermentation extracts [14]. Subsequently, both compounds were isolated from *Cylindrocladium ilicicola* strain MFC-870 and named ilicicolin A and ilicicolin B, respectively [15, 16]. Both compounds were also discovered in several filamentous fungi of different genus, such as *Acremonium, Cylindrocarpon, Nectria*, and *Neonectria* [17–24].

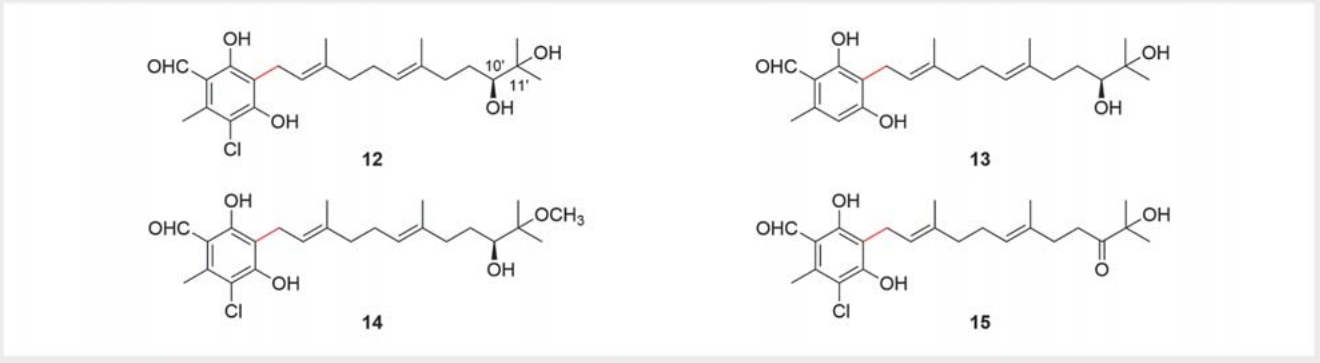
Compound **1** can inhibit the proliferation of lymphocytic leukemia Jurkat cells at 10 μM with an inhibition rate of 71.43% [25]. Guo et al. reported that **1** showed an antitumor effect by inhibiting the signal pathway of enhancer of zeste homolog 2 [26]. Compound **2** showed testosterone-5α-reductase (T-5α-reductase) inhibitory activity on rat prostate with an IC_{50} value of 0.36 mM [20] and antitrypanosomal activity toward *Trypanosoma brucei brucei* strain GUTat 3.1 and *Trypanosoma brucei rhodesiense* strain STIB900 with IC_{50} values of 49 and 59 nM, respectively [27]. T-5α-reductase is one of the important drug targets for benign prostatic hyperplasia by inhibiting the transformation of testosterone to dihydrotestosterone [28–31]. Compound **2** also exhibited antibacterial activity against *Bacillus subtilis* and methicillin-resistant *Staphylococcus aureus* (MRSA) with IC_{50} values of 1.06 and 0.74 μM, respectively [23], and moderate antifungal activity against *Ustilago violacea and Fusarium oxysporum* with inhibition zones of 4 and 5 mm [32].

LL-Z 1272α epoxide (**3**) (▶ **Fig. 2**) was isolated as a precursor of ascochlorin from *A. viciae* J-29 in 2009 [33]. Compound **3** was the epoxidation product of **1**. Both *Microcera* sp. BCC 17074 and *A. sclerotigenum* GXIMD 02501 produced **3** [18, 34]. It showed weak cytotoxic activity and potent hDHODH inhibition with an IC_{50} value of 1.6 μM [18].

Ilicicolinic acid A (**4**) (▶ **Fig. 2**) and ilicicolinic acid B (**5**) (▶ **Fig. 3**) were first isolated from *Cylindrocarpon* sp. in 1993 [35]. Afterward, chemical studies were performed on *Neonectria discophora* SNB-CN63, which led to the isolation of ilicicolinic acids A, C–G (**6**–**10**), and ilicicolinol (**11**) (▶ **Fig. 3**) [19, 36]. According to the Markovnikov orientation, compound **6** might be an H_2O addi-



▶ **Fig. 3** Structures of compounds **5–11**.



▶ **Fig. 4** Structures of compounds **12–15**.

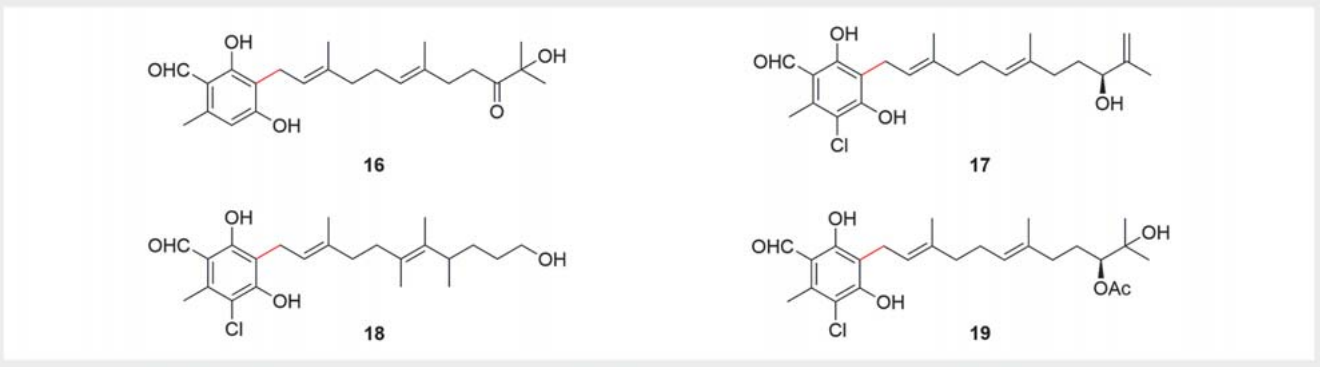
tion product of **4**. However, C-7′ configurations of **8** and **9** were unassigned.

Antimicrobial activities of compounds **4–11** were evaluated. Compound **4** was found to be selective activity with a minimum inhibitory concentration (MIC) value of 1 µg/mL against *Escherichia coli*. (ATCC 25 922). Compounds **8** and **9** were found to be selective in activity against microbial pathogen *Trichophyton rubrum* SNB-TR1 with MIC values of 8 and 8 µg/mL. Compounds **6, 7**, and **11** showed weak antimicrobial activity against *T. rubrum* SNB-TR1, *S. aureus* ATCC29213, and MRSA ATCC33591, while compound **10** exhibited no antimicrobial activity (MIC>128 µg/mL) [19, 36]. Comprehensive analysis of the structure-activity relationships suggested that the presence of the chlorine atom was important to exert antimicrobial activities.

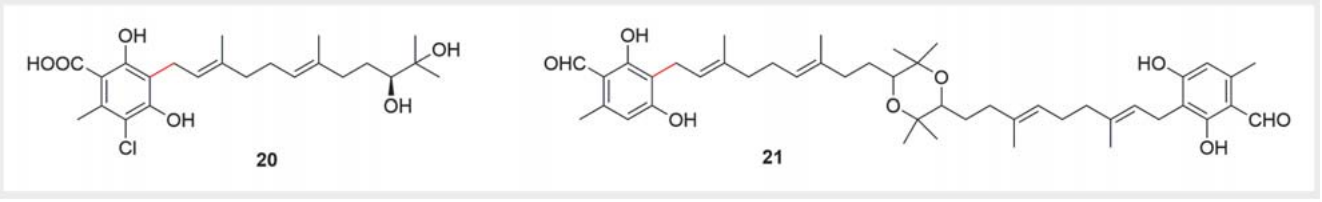
Chlorocylindrocarpol (**12**) (▶ **Fig. 4**) was discovered from marine sponge-derived *Acremonium* sp. in 2009 [37, 38]. Cylindrocarpol (**13**) (▶ **Fig. 4**) was isolated from *Cylindrocarpon lucidum* (MF 5710) in 1996 [39]. Subsequently, **12** and **13** were also found in *Acremonium* sp., *Microcera* sp. BCC 17 074, and *A. sclerotigenum* GXIMD 02 501 [18, 34, 37]. Compound **12** exhibited stronger antitumor activity against the MCF-7 cell line (breast cancer) with an IC_{50} value of 6.2 µg/mL, comparable to doxorubicin (IC_{50}=8.6 µg/mL) [34]. It also displayed weak anti-inflammatory activity to inhibit the production of NO and IL-6 [37]. Compound **13** showed weak inhibitory activity (MIC=77 µM) against farnesyl-protein transferase (FPTase), which is a potential target for anticancer drugs [39]. Recently, **12** and **13** are reported to show hDHODH inhibitory activity with IC_{50} values of 3.7 and 5.0 µM, respectively [18]. Nectchlorin B (**14**) (▶ **Fig. 4**) was a methoxy adduct from compound **12**. It was isolated from *Microcera* sp. BCC 17 074 and showed weak cytotoxicity [34].

Acremochlorins I–M (**15–19**) (▶ **Fig. 4** and **5**) were identified as hDHODH inhibitors from coral-derived fungus *A. sclerotigenum* GXIMD 02 501. They showed hDHODH inhibition with the IC_{50} values of 9.3, 7.9, 0.39, 0.50, and 0.52 µM, respectively. They also exhibited potent antiproliferative activity against MDA-MB-231 and MDA-MB-468 cell lines, with the IC_{50} values ranging from 1.7 to 12 µM, except **15** and **16** (IC_{50}>60 µM) [18].

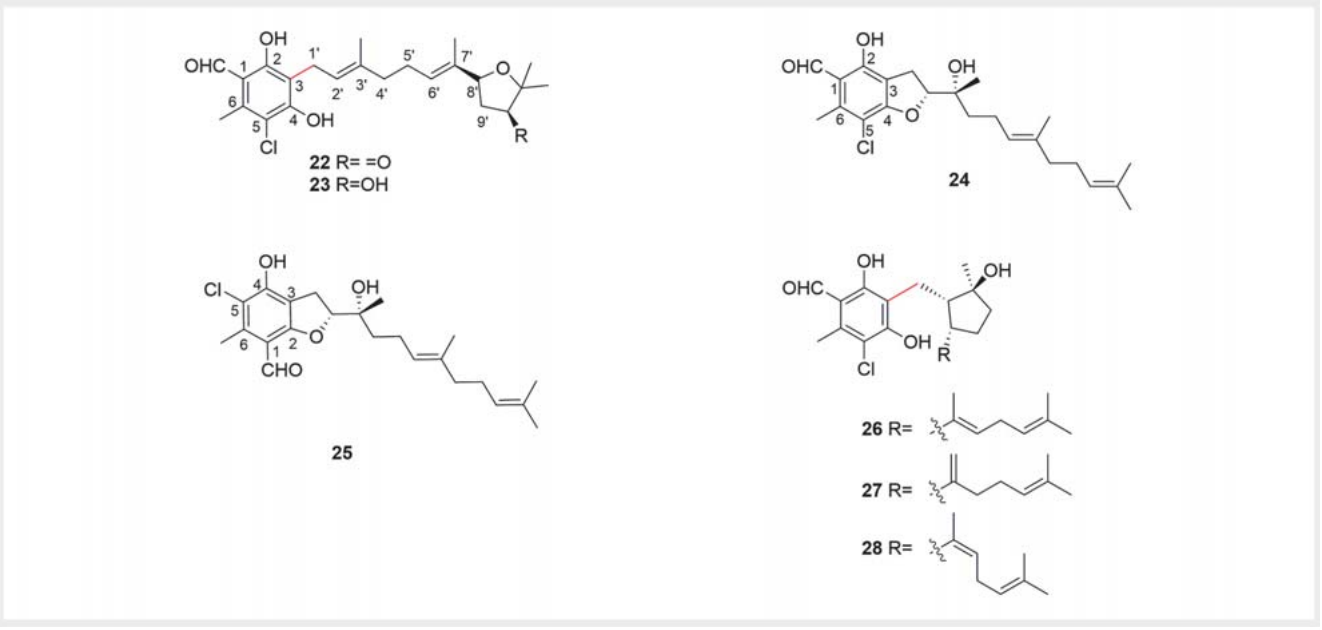
10′-hydroxyilicicolinic acid D (**20**) (▶ **Fig. 6**) was initially isolated from *Cylindrocarpon* sp. SY-39 in 2018 and displayed antimicrobial activity against *S. aureus* (MIC=5.0 µg/mL) [40]. Cylindrocarpol dimer (**21**) (▶ **Fig. 7**) was reported as a fungal metabolite, but its origin had not been identified; **21** showed hDHODH enzyme inhibition with an IC_{50} value of 2.03 µM and anti-proliferation effects toward T lymphocyte cells with an IC_{50} value of 9.32 µM [41].



▶ **Fig. 5** Structures of compounds **16–19**.



▶ **Fig. 6** Structures of compounds **20** and **21**.

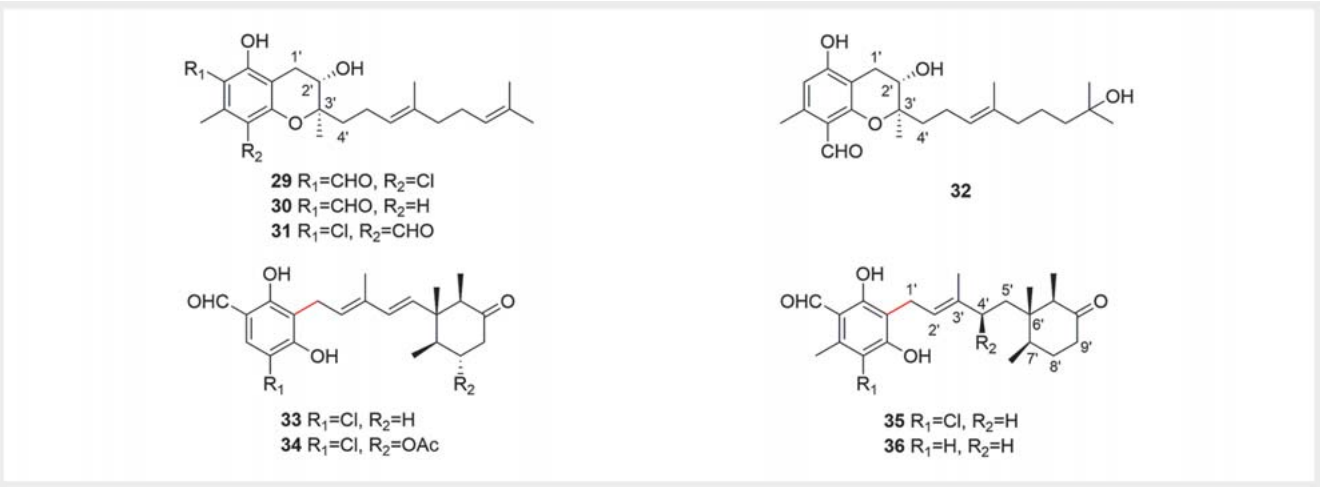


▶ **Fig. 7** Structures of compounds **22–28**.

Monocyclic Type

Most of these groups are distributed in monocyclic type, in which the farnesyl group was formed to the monocyclic sesquiterpene scaffold. In the group, a variety of cyclization patterns and post-modifications increased greatly the structure diversity. A total of 46 molecules (**22–67**) are included in the group, and **33** represents the first case of the group. Abundant cyclization patterns are endowed with significant activities.

Ascofuranone (**22**) (▶ **Fig. 7**) was initially isolated from *A. viciae* in 1972 [38, 42]. Afterward, compound **22** appeared again in *Acre-*



▶ **Fig. 8** Structures of compounds **29–36**.

monium sp., *Paecilomyces variotii* INA-199, *Verticillium hemipterigenum* BCC 2370, and *Nectria* sp (HIL Y 90 3333) [37,43–47]. The detailed and impressive bioactivities will be discussed below.

Ascofuranol (**23**) (▶ **Fig. 7**) was discovered from *A. viciae* Libert in 1973 [48] and coexisted with its derivatives in several filamentous fungi such as *Acremonium* sp. (J05B-1-F-3), *A. sclerotigenum* GXIMD 02501, and *V. hemipterigenum* BCC 2370 [18,37,44]. Compound **23** exhibited weak anti-inflammatory activity [37] and hDHODH inhibition with an IC_{50} value of 0.72 µM, as well as moderate cytotoxic activity against MDA-MB-231 and MDA-MB-468 cell lines with IC_{50} values of 12 and 11 µM, respectively [18].

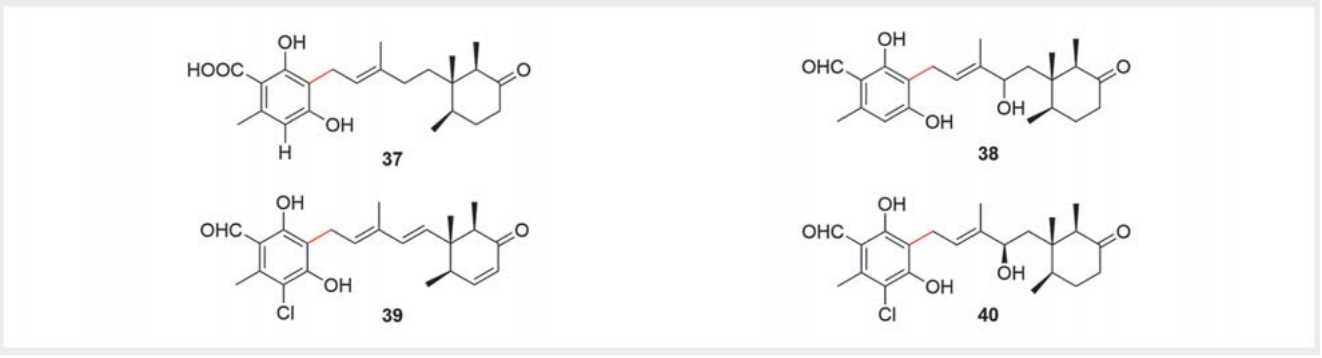
Ilicicolinal derivatives, ilicicolinal (**24**), and ilicicolinals B–I (**25–32**) (▶ **Fig. 7** and **8**), together with compounds **4–11**, were obtained from *N. discophora* SNB-CN63 [19]. Structurally, **24** and **25** are isomers; the furan ring in **24** is formed by the ether bond between C-3 and C-4, while that of **25** is due to etherification between C-2 and C-3. Compounds **26–28** possess a five-membered ring-containing sesquiterpene scaffold. Compounds **29–30** have a pyranoid ring formed between C-3 and C-4, while the formation of the pyranoid ring in **31** and **32** occurs between C-2 and C-3. Antimicrobial activities studies showed that **25–27** and **32** were selectively active with MIC values ranging from 8 to 16 µg/mL on two pathogens, *S. aureus* ATCC29213 and MRSA ATCC33591, while **24** and **28–31** were inactive (MIC≥128 µg/mL) [19]. Comparison of **24** and **25** suggests a formation pattern of ether was crucial for the antimicrobial activity.

Ascochlorin (**33**) (▶ **Fig. 8**) was originally purified from *A. viciae* in 1968, and the partial structure was determined [4]. Its structure was finally elucidated by a combination of X-ray crystallography and physicochemical methods in 1969 [49]. Subsequent metabolite ilicicolin D (*C. ilicicola* MFC-870) and LL-Z 1272γ (*Fusarium* sp. LL-Z 1272) were confirmed to be identical to ascochlorin by comparisons of NMR, IR, TLC, and mixed melting point determination [14–16,21].

Compounds **22** and **33** are representative molecules of the hybrids and exhibited impressive and diverse bioactivities, which have been well-documented in previous reviews [5,7,10,37,48,50–87]. Gao et al. reviewed antitumor activity, mechanisms, and antitumor drug targets of compounds **22** and **33** in 2020 [7]. As reviewed, **22** is a unique target for the treatment of African trypanosomiasis [10,85,88–94]. Briefly, compound **22** was found to be an inhibitor of TAO ubiquinol oxidase [54], recombinant *Trypanosoma vivax* alternative oxidase with a K_i value of 0.40 nM, and rTAO inhibitor with an IC_{50} value of 1.3 nM [66,69,95–104]. Potent inhibitory activity of trypanosomal glycerol kinase and TAO of *T. b. brucei* for **22** were described in 2019 [105]. In addition, **22** and **33** showed inhibitory activity against *Cryptosporidium parvum* AOX (alternative oxidase) with IC_{50} values of 0.3 and 500 nM, respectively [68], potent sensitivity of recombinant *Sauromatum guttatum* AOX with IC_{50} values of 0.06 and 7 nM and native *Arum maculatum* AOX with IC_{50} values of 0.16 and 70 nM [106–108]; **33** was also reported to show specific inhibitory effect toward mitochondrial cytochrome bc1 complex [109] and a recombinant AOX inhibitor expressed in *E. coli* membranes 7.4 ± 3 nM [110].

Compounds **22** and **33** also showed antimicrobial activities. Both compounds exhibited moderate fungicidal activity against plant pathogenic fungus *Phytophthora infestans* [43], as well as significant antimicrobial activity against *S. aureus*, MRSA, *S. epidermidis*, *B. subtilis*, and the human pathogenic fungus *Candida albicans* with MIC values of 0.25–32 µg/mL [22,47,111]; **33** was active against *Pseudomonas syringae* with an IC_{50} value of 28.5 µM [112] and against *Aspergillus fumigatus* with MIC values of 1.25 to 2.5 µg/mL [113,114]. Furthermore, **22** and **33** displayed anti-inflammatory activity [37,115–121], as well as a potent agonist of peroxisome proliferator-activated receptor with IC_{50} values of 3.2 and 1 µM, respectively [122].

LL-Z 1272ζ (**34**), LL-Z 1272δ (**35**), and LL-Z 1272ε (**36**), together with **1, 2,** and **33,** were isolated from *Fusarium* sp. LL-Z 1272 in 1969 (▶ **Fig. 8**) [14,38]. *Verticillium* SP FO-2787, *Fusarium* sp. (internal strain 3042), *Acremonium* sp., *Cylindrocarpon* sp. FKI-4602, etc. are additional producers of these hybrids [21,22,34,37,43,44]. It was noted that LL-Z 1272ζ (**34**) was proven to be the same as ilicicolin F.; **35** was known as ilicicolin C and 4,5-dihydroascochlorin, while **36** was identical to 4,5-dihydrodechloroascochlorin



▶ **Fig. 9** Structures of compounds **37–40**.

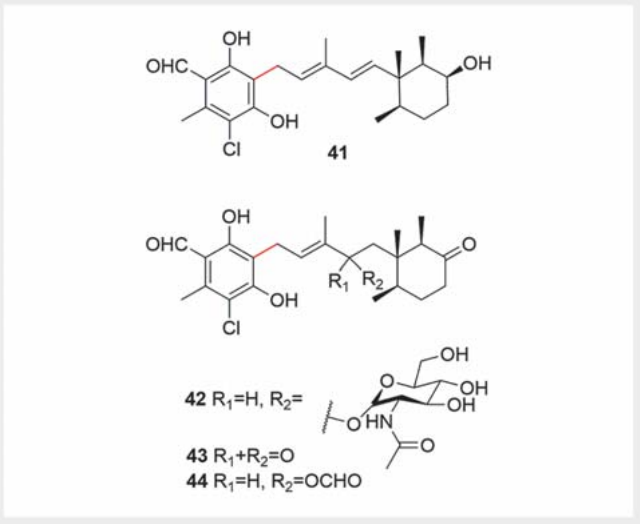
[15, 16, 21, 32]. Cyclohexanone-containing sesquiterpene moiety is the most common scaffold in the family, which is usually cyclized by the relevant terpene cyclase.

The bioactivities of **34–36** were evaluated. All exhibited moderate T 5α-reductase inhibitory activity with IC_{50} values of 0.37 mM, 0.34 mM, and 0.37 mM, respectively [20]. Furthermore, **34** showed potent fungicidal activity against plant pathogenic fungi *P. infestans* at 500 mg/L [43] and good cytotoxic activities toward three cancer cell lines, KB, BC-1, and NCI-H187, as well as Vero cells with IC_{50} values ranging from 0.69 to 1.9 μg/mL [44]; **34** was also active with IC_{50} values of 28.5 μM against *P. syringae* [112]. Compound **35** could selectively inhibit the production of NO at 100 μM [37] and potent antibacterial activity toward *B. megaterium* with a zone of 10 mm [32]. Compound **36** showed strong antifungal activity toward *Eurotium repens* with a zone of 15 mm [32].

Another two derivatives, LL-Z 1272ε acid (**37**) and dechlorodeacetyloronctrin (**38**), were first separated and purified from *Nectria* sp. B-13 (▶ **Fig. 9**). Compound **37** can completely inhibit the spore germination of *Magnaporthe grisea* with an MIC value of 15.6 μg/mL. Compound **38** had a weak inhibitory effect on the activity of *Pyricularia oryzae* [123].

Cylindrochlorin (**39**) (▶ **Fig. 9**), also named 8′,9′-dehydroascochlorin [43] and ilicicolin E [15, 16], was first discovered from the methanol extract of the mycelium of *Cylindrocladium* sp. in 1970 and showed antiviral activity against Newcastle disease virus [124]. Meanwhile, compound **39** was found to be active on herpes simplex virus type 1 (HSV-1) with an IC_{50} value of 0.19 μg/mL [44]. It showed potent fungicidal activity against *A. fumigatus* with an MIC value of 4.1 μM [43, 114]. It also exhibited cytotoxic activities in KB, BC-1, NCI-H187, and Vero cells with IC_{50} values of 2.4, 0.53, 1.3, and 0.69 μg/mL, respectively [44]. Compound **39** was subsequently found from *Cylindrocarpon* sp. FKI-4602, *Nectria* sp (HIL Y 90 3333), *Stilbella fimetaria* (IBT 28361), and *Verticillium* SP FO-2787 [20, 22, 43, 114].

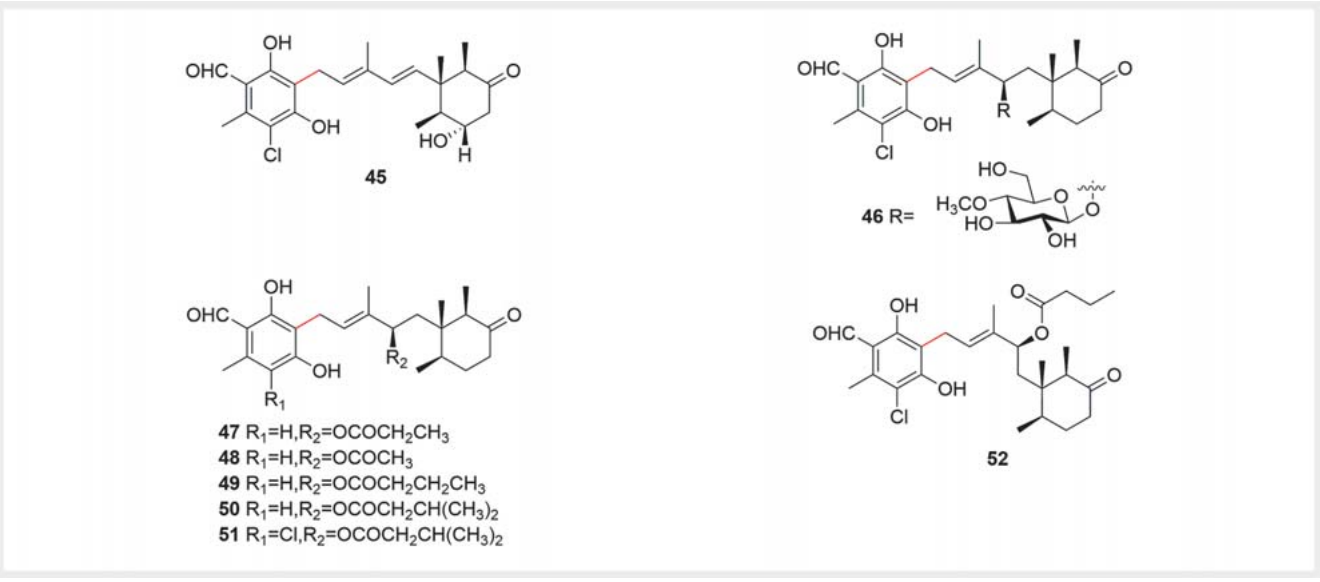
Bioactivity-guided fractionation led to the discovery of hypolipidemic 4′-hydroxy-5′-hydroascochlorin (**40**) from *A. viciae* Libert in 1974 (▶ **Fig. 9**) [125], while 4′,5′-dihydro-4′-hydroxyascochlorin, deacetylchloronectrin, and 4′,5′-dihydro-4′β-hydroxyascochlorin are considered to have the same structure with 4′-hydroxy-5′-hydroascochlorin [37, 44, 45, 47, 114]. Compound **40**



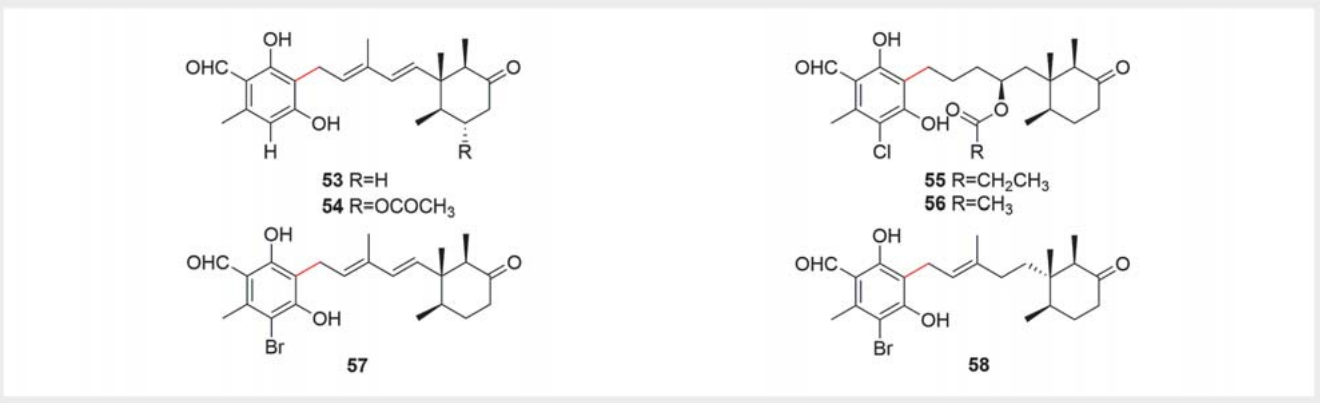
▶ **Fig. 10** Structures of compounds **41–44**.

was usually co-isolated with 10′-deoxy-10′α-hydroxyascochlorin (**41**), ascofuranone (**22**), ascochlorin (**33**), and other derivatives from different fungal genus, such as *Acremonium* sp. LG0808, *V. hemipterigenum* BCC 2370, *Stilbella fimetaria* (IBT 28361), etc. [37, 44, 45, 47, 113]. Compounds **40** and **41** showed potent cytotoxic activities against the A549 (lung cancer) with IC_{50} values of 4.1 and 0.9 μM, and the HepG2 (hepatocellular carcinoma) cancer cell line with IC_{50} values of 44.7 and 5.8 μM, respectively [47]. Moreover, **40** can selectively inhibit NO and IL-6 production to display anti-inflammatory activity [37] and showed strong antibacterial activities against *Ralstonia solanacearum* with an MIC value of 3.13 μg/mL [126]; **41** exhibited metastatic prostate cancer cell migration inhibitory activity at 6 μM [45] and weak antimicrobial activity [47].

Ascochlorin *N*-acetylglucosamine (**42**), 4′-ketoascochlorin (**43**), and 4′5′-dihydro-4′-formylascochlorin (**44**) were isolated from marine-derived fungus *S. fimetaria* IBT 28361 by the targeted dereplication of fungal extracts via UHPLC-DAD-QTOF-MS (▶ **Fig. 10**). The additional amino sugar unit at the C-4′ position of **42** was identified as N-acetyl-α-D-glucosamine. At present,



▶ **Fig. 11** Structures of compounds **45–52**.



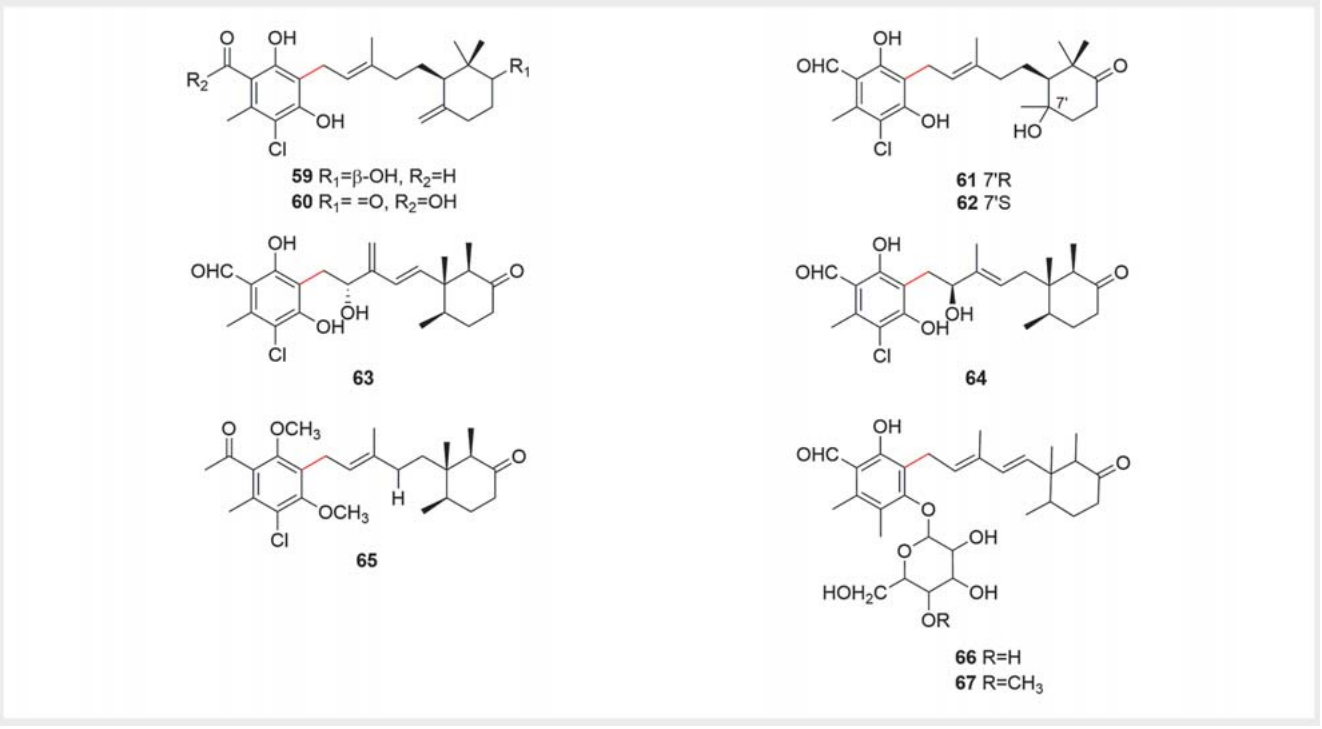
▶ **Fig. 12** Structures of compounds **53–58**.

only compound **42** and vertihemipterin A (**46**) have α-D-glucosamine unit. Antifungal and antibacterial activities were assessed; **43** was found to have weak antibacterial activities against MRSA, while others were inactive [114].

8′-hydroxyascochlorin (**45**) (▶ **Fig. 11**), vertihemipterin A (**46**), 4′,5′-dihydro-4′-hydroxyascochlorin, and 8′,9′-dehydroascochlorin were separated from the extracts of *V. hemipterigenum* BCC 2370 [44]. Meanwhile, compound **45** possessed more potent cytotoxicity than **46** [44].

In 1996, Singh et al. reported that cylindrol A (**47**), cylindrols A_1-A_4 (**48–51**), and cylindrols B and B_1 (**53–54**) were obtained from *C. lucidum* (MF 5710, ATCC 74261) (▶ **Fig. 11** and **12**) [38, 39, 127]. A soil-derived fungus, *Cylindrocarpon* sp. FKI-4602, was also reported to produce cylindrol A_5 (**52**), cylindrol A_4 (**51**), and cylindrol B (**53**) [22]. Compounds **47, 48, 50–51**, and **53** showed broad FPTase inhibitory activity with IC_{50} values of 0.7 μM to 13 μM [39]. In addition, **52** exhibited moderate antimicrobial activities toward *B. subtilis* ATCC 6633, *Acholeplasma laidlawii* KB174, *M. smegmatis* ATCC 607, and *Kocuria rhizophila* ATCC 9341 [22]; **53** could inhibit the production of NO, TNF-R, and IL-6 at 100 μM [37].

Chloronectrin (**55**) (▶ **Fig. 12**) was initially discovered from *Nectria coccinea* in 1972 [21]. Nectchlorin A (**56**) (▶ **Fig. 12**), together with **55** and **14**, were isolated from *Microcera* sp. BCC 17074 [34]. The five carbon linkers in **55** and **56** are further oxidized in contrast to analogs, and they displayed weak cytotoxic activity [34]. When bromide was added to chloride-free medium, *Fusarium* sp. could produce the bromo-analogue of ascochlorin, 3-bromoascochlorin (**57**) [21]. Supplementation of KBr to the *Fusarium* sp. culture medium led to the production of 3-bromoascochlorin (**57**) and 3-bromo-12,13-dihydroascochlorin (**58**) (▶ **Fig. 12**). Compound **58** (IC_{50}=17.8 μM) showed slightly stron-



▶ **Fig. 13** Structures of compounds **59–67**.

ger cytotoxicity than compound **57** (IC_{50}=21.3 μM) [128]. Zhang et al. studied the antitumor mechanism of compound 57 by suppressing the MAPK pathway [129].

Acremochlorin A–F (**59–64**) (▶ **Fig. 13**), together with compounds **15–19**, were isolated from coral-derived fungus *A. sclerotigenum* GXIMD 02501. Among them, **59** showed potent hDHODH inhibition with the IC_{50} values of 74 nM and cytotoxic activity against MDA-MB-231 and MDA-MB-468 cell lines, with IC_{50} values of 0.65 and 0.48 μM. Other metabolites (**60–64**) showed moderate hDHODH inhibition with IC_{50} values ranging from 3.9 to 34 μM and weak cytotoxic activities (IC_{50}>60 μM) [18].

DimethoxyilicicolinC (**65**) (▶ **Fig. 13**) was initially derived from *Nectria* sp. B-13 by silica gel column, gel chromatography, reverse phase silica gel chromatography, preparation thin-layer chromatography (PTLC), and HPLC [25]. It can be regarded as the methoxy substituent of ilicicolin C. Another difference is that compound **65** has an additional methyl group at C-8. However, compared with other analogs, it has no biological activity. In 1997, TAN-2355A (**66**) and TAN-2355B (**67**) (▶ **Fig. 13**) with glycosides were found from *Acremonium* sp. FL-65227. For the binding of [3H]-TRH to CHO cells, compound **66** showed inhibitory activity with an IC_{50} value of 52 μM [38, 130].

Bicyclic Type

This class contains four molecules (**68–71**) that are featured by the farnesyl group formed to a bicyclic sesquiterpene scaffold. In detail, compound **68** has a furan ring and a cyclohexanone moiety; **69** possesses a decalin unit and both metabolites **70** and **71** are involved in a fused 13–5 bicyclic ring system (▶ **Fig. 14**).

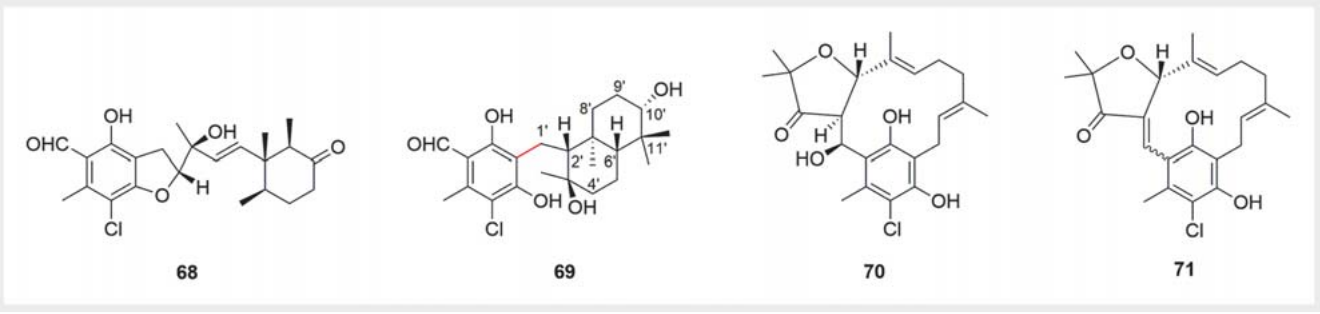
Acremochlorin G (**68**) and acremochlorin H (**69**) were co-isolated with compounds **59–64** from the coral-derived fungus *A. sclerotigenum* GXIMD 02501 and showed weak cytotoxic activity against MDA-MB-231 and MDA-MB-468 cells and hDHODH inhibitory activity. Interestingly, compound **69** is the first case of the ASC-type meroterpenoids with a drimane unit [18].

Acremofuranone A (**70**) and acremofuranone B (**71**) were isolated from *Acremonium* sp. (J05B-1-F-3) [37, 38], with the unprecedented cyclic skeleton. Compound **71** was defined as a dehydrated derivative of **70**.

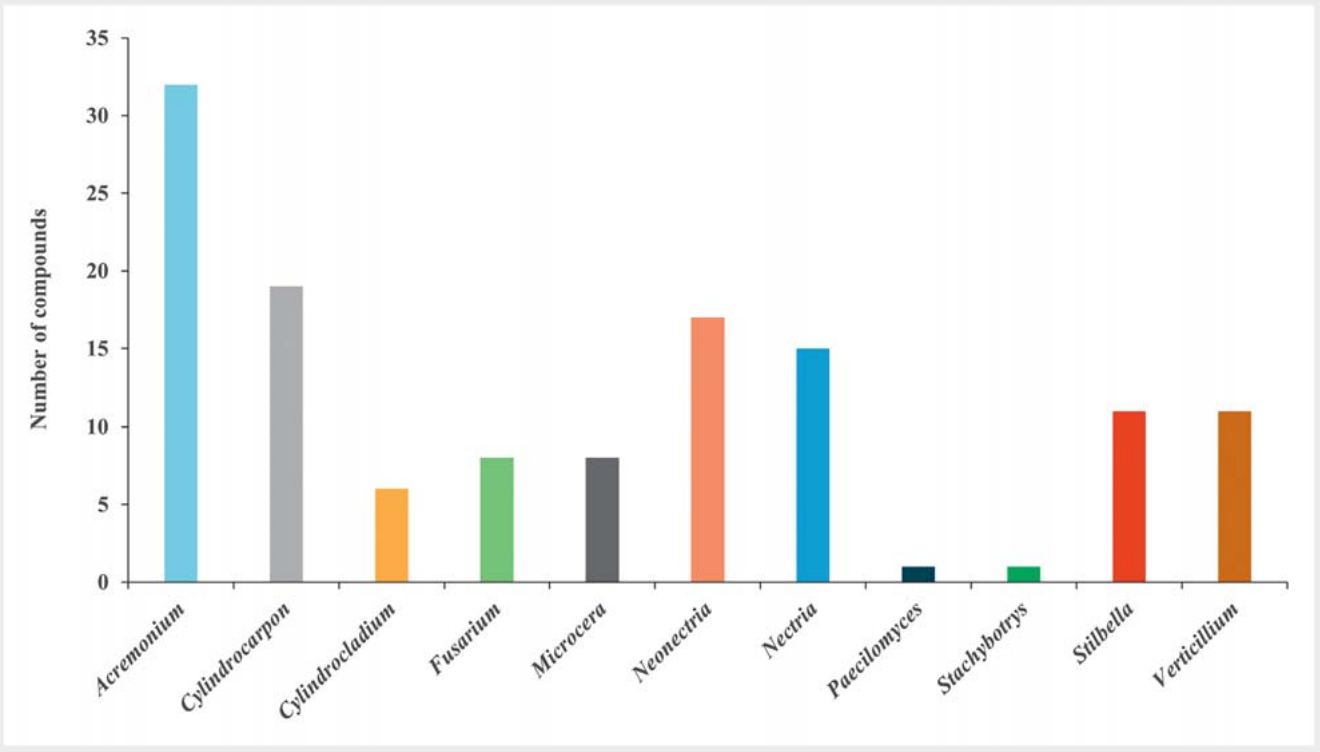
Conclusion

Orsellinic acid-sesquiterpene meroterpenoids aroused the great interest and attention of scientists because of their diverse bioactivities, particularly antitrypanosomal activity and antitumor activity inhibiting the hDHODH target protein. This review summarizes structures, filamentous fungi sources, activities, and biosynthesis of these types of compounds from 1968 to June 2022.

The statistical data revealed most orsellinic acid-sesquiterpenoid hybrids are produced by filamentous fungi. Of all the compounds reviewed, *Acremonium*/*Ascochyta* (25%), *Neonectria* (14%), *Cylindrocarpon* (14%), *Fusarium* (11%), *Verticillium* (7%), and *Stachybotrys* (7%) are predominant producers of increased structural diversity (▶ **Fig. 15**). The remaining 22% of these compounds are scattered across another four genera including *Stilbella, Microcera, Nectria*, and *Cylindrocladium.* Furthermore, considering the structure frameworks, nearly 48% of the linear type, 39% of the monocyclic, and 100% of the bicyclic type are derived from



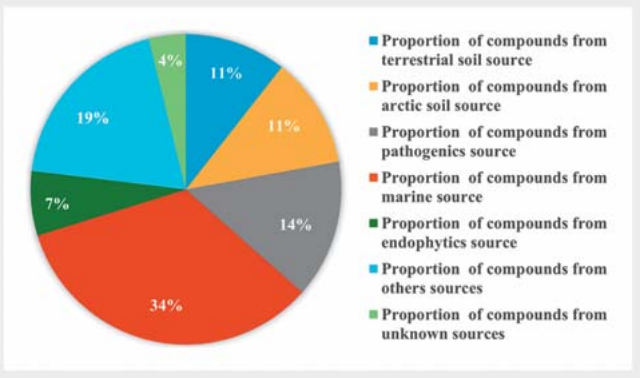
▶ **Fig. 14** Structures of compounds **68–71**.



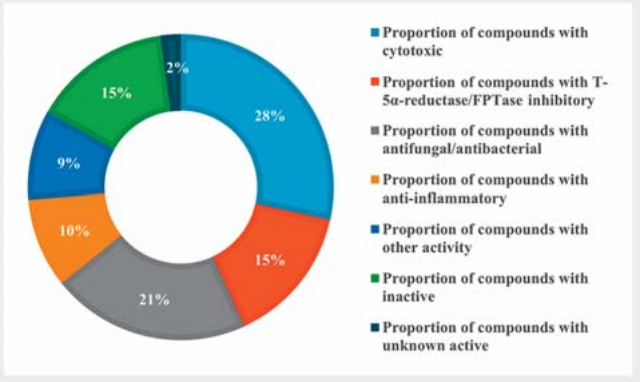
▶ **Fig. 15** Fungal genus distribution of isolated compounds.

Acremonium spp., indicating the potential differences of gene constitution in gene cluster or/and transcriptional regulation between different genera. Maybe it results from secondary metabolism regulated by environmental factors such as pH, medium nutrition, culture temperature, etc. In terms of inhabiting ecological niches, 34% of the investigated hybrids are produced by marine-derived fungi that are isolated from coral, sponge, and driftwood; 22 percent, 14 percent, and 7 percent of these compounds are coming from terrestrial soil- or arctic soil-derived fungi, pathogenic fungi, and endophytic fungi, respectively. In addition, dried cow dung, the dead leaf of beech, and a *Nasutitermes corniger* termite aerial nest-derived fungi are also the potential producers for production of the hybrids (19%), as shown in ▶ **Fig. 16**. It is noted that the hybrids produced by pathogenic fungi may play an important role in infecting the host or chemical arsenals for defense and even in the area of biological control.

The related bioactivities for the meroterpenoids were also discussed in this review. To sum up, they showed a wide range of biological activities (▶ **Fig. 17**). Approximately 28% of the meroterpenoids possessed cytotoxic activity including antitumor and antiproliferative activities. About 21%, 15%, and 10% of the meroterpenoids exhibited antimicrobial activity, testosterone-5α-reductase or farnesyl-protein transferase inhibitory activity, and anti-inflammatory activity, respectively (▶ **Fig. 17**). The remaining 9% showed other activities including antiviral, hypolipidemic, and antitrypanosomal activities. Specifically, com-



▶ **Fig. 16** Diverse ecological environments of producing fungi.



▶ **Fig. 17** Bioactivity distribution of isolated compounds (**1–71**).

pound **2** showed more potent anti-African-trypanosomiasis activity (IC_{50}= 0.049–0.059 μM) than fexinidazole (IC_{50}=0.7–3.3 μM). The fexinidazole is the only oral preparation for the treatment of human African trypanosomiasis approved by the FDA; thus, compound **2** could be a potential anti-African-trypanosomiasis drug candidate or lead structure. Compound **59** showed stronger DHODH inhibitory activity (IC_{50}=74 nM) than teriflunomide which was approved by FDA for treating multiple sclerosis and rheumatoid arthritis. Furthermore, compound **59** has higher safety and fewer side effects than the commonly used DHODH inhibitor brequinar, suggesting the meroterpenoids are promising molecules for discovering the new class of DHODH inhibitors. Considering the lack of a systematic bioactive evaluation of these compounds in unified conditions, we failed to summarize the structure-activity relationships of the meroterpenoids among the linear, mono-, and bicyclic types. However, we take DHODH inhibition as an example; the bicyclic sesquiterpenes are less effective than that of linear and monocyclic sesquiterpenes. Most linear sesquiterpenes have better DHODH inhibitory activity than monocyclic sesquiterpene. Obviously, rich cyclization patterns about farnesyl moiety may have an impact on hDHODH inhibition.

Given the aforementioned potential pharmaceutical values of the meroterpenoid hybrids, accurate mining of these resources is sought after. In the post-genome era, bioinformatics and omics analysis will facilitate the location of related biosynthetic gene clusters in the fungi kingdom. While using genome mining technologies, complemented by the global natural products, molecular networks (GNPs) can accelerate the discovery of the resource of the unknown natural hybrids. Considering silent biosynthetic gene clusters, low expression in the host strains, and the need for structure derivatization, the synthetic biology method and combinatorial biosynthesis, which are based on a platform of heterologous expression, will provide the opportunities to rationally access unidentified natural products and natural-product-like molecules.

Supporting Information

Filamentous fungi sources and biological activities of orsellinic acid-sesquiterpene hybrids from 1968 to June 2022 are available as Supporting Information.

Contributors' Statement

Literature collection: H. Gao, L. Zhou, Y. Wang, X. Qian, Y. Liu; manuscript framework construction: H. Gao, P. Zhang, G. Wu; structural painting and proofreading: X. Qian, Y. Liu, Y. Wang; original draft writing: H. Gao, G. Wu; critical revision, language polishing, optimization of the manuscript: H. Gao, L. Zhou, P. Zhang, Y. Wang, G. Wu.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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