Endolichenic fungi: a new source of rich bioactive secondary metabolites on the horizon

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Abstract:

Endolichenic fungi are diverse groups of predominantly filamentous fungi that reside asymptomatically in the interior of lichen thalli. Natural products from endolichenic fungi, isolated from a variety of different lichen species, have been attracting increased attention for their potential to produce bioactive metabolites possessing new structures and representing different structural classes. This is evident from the steady increase of publications devoted to endolichenic fungal metabolites over the past decade, since the first report of endolichenic secondary metabolites. The bioactive metabolites produced by endolichenic fungi originate from multiple biosynthetic pathways and occupy different chemical structure classes, including steroids, quinones, terpenoids, peptides, xanthones, sulfur-containing chromenones, etc. Endolichenic fungal metabolites possess a diverse array of bioactivities, such as anticancer, antiviral, antibacterial, antifungal, and anti-Alzheimer's disease. This review provides the first thorough assessment of endolichenic fungi, their biodiversity, secondary metabolites, and associated bioactivity. This review will highlight the bioactive metabolites reported in recent years from endolichenic fungi, as well as discussing the potential of these symbiotic fungi as sources of new, diverse natural products with varying bioactivities.

Keywords: Bioactivity | Biodiversity | Endolichenic fungi | Lichen | Natural products

Article:

Abbreviations

AD	Alzheimer's disease
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FIC	Fractional inhibitory concentration
GPS	Global positioning system
IC_{50}	50 % inhibitory concentration

ITS	Internal transcribed spacer
MEA	Malt extract agar
MIC	Minimum inhibitory concentration
OSMAC	One-strain, many compounds
PDB	Potato dextrose broth

Endolichenic fungi

Fungal strains represent a rich source of biologically active natural product metabolites with wide-ranging biological activity. Although investigations into fungal metabolites date back to the 1870 s, the first systematic survey of fungal metabolites wasn't initiated until after World War I by Harold Raistrick (1949). Since those initial forays into fungal secondary metabolites, natural products from fungal sources have been employed as pesticides, herbicides, antibiotics, immunosuppressants, anti-infectives, and anticancer agents (Hoffmeister and Keller 2007). And yet, it is believed that only about 5 % of the global fungal species have been identified (Young 1997). Fungi occupy a wide diversity of environmental and ecological niches across the globe, including terrestrial, fresh water, and marine environments where they function as saprobes, symbionts, and pathogens. Groups of highly diverse fungi reside within the internal tissue of other organisms, living asymptomatically without any obvious sign of infection. Endophytic fungi occur within tissues of host plants and are dominated by ascomycetous fungi. Endophytic fungi have received increased attention as sources of natural products, especially after the discovery of paclitaxel (taxol) in the endophytic fungus *Taxomyces andreanae*, which inhabits the original source of the important anticancer drug, *Taxus brevifolia* (Stierle et al. 1993). Multiple reviews have highlighted the metabolite diversity and potential of endophytic fungi to produce pharmaceutically valuable natural products (Kaul et al. 2012; Nisa et al. 2015; Proksch et al. 2010; Strobel et al. 2004; Tan and Zou 2001). An analogous group of fungi inhabit the thalli of lichens in a similarly asymptomatic manner: the endolichenic fungi.

Lichen thalli are an emergent property arising due to symbiotic association between a fungal organism (mycobiont) and at least one chlorophyll-containing photosynthetic organism (photobiont) such as a micro alga, a cyanobacterium, or both (Lutzoni and Miadlikowska 2009). In addition to the mycobiont of the lichen, the thallus is usually home to numerous, asymptomatic, cryptic microfungi that live in close association with the photobiont (Arnold et al. 2009). These diverse groups of fungi, which reside in the interior of a lichen thallus, have been termed as 'endolichenic fungi' (Arnold et al. 2009; Miadlikowska et al. 2004). Endolichenic fungi were discovered when attempts were being made to isolate the lichen forming mycobiont into pure culture (Crittenden et al. 1995; McDonald et al. 2013; Petrini et al. 1990). These fungi are similar to the endophytic fungi (sometimes also referred to as endophyte-like fungi) (Arnold et al. 2009; U'Ren et al. 2016), which reside within healthy tissues of plants and are a phylogenetically and ecologically diverse without causing any disease symptoms (Arnold 2001, 2007; Petrini 1991). The endolichenic fungi, however, are distinct from

mycobionts (Lutzoni and Miadlikowska 2009), which make up the lichen thallus, and from lichenicolous fungi, an ecological group of meiosporic and mitosporic fungi that can often be observed on living lichens (Arnold et al. 2009). The endolichenic fungi consist of mostly horizontally transmitted, functionally advantageous fungi, and include abundant taxa belonging to diverse classes, orders and families within the Ascomycota (Pezizomycotina) (Arnold et al. 2009; Girlanda et al. 1997; Kannangara et al. 2009; Li et al. 2007; Petrini et al. 1990; Suryanarayanan et al. 2005; Tripathi and Joshi 2015; U'Ren et al. 2010, 2012). Endolichenic fungi have, of late, become a new avenue for evaluation of bioactive secondary metabolite chemistry in natural products research.

Cultures of endolichenic fungi over the last 10 years have revealed potential new structures, and interest in their production of bioactive natural products has increased substantially. Since metabolites from endolichenic fungi were first reported 9 years ago, research into endolichenic fungal natural products has steadily increased, representing a small but growing body of literature (Fig. 1). Thus, the focus of the current review is on the progress made over the last decade by natural product chemists and their mycology collaborators in isolating new secondary metabolites from endolichenic fungi and their associated bioactivity. Containing over 140 novel metabolites, this review is the first to summarize the biodiversity, metabolites, and bioactivity of natural products derived from fungi that live in symbiosis with lichens. This review covers the literature available in SCOPUS (http://www.scopus.com/) up through December 2015; included articles were found using the open text string "endolichen*". This review is timely given that there has been a sudden surge in the natural products literature on isolation of bioactive chemical compounds from endolichenic fungi and there is a critical need for synthesis of the literature from the numerous studies that have been published thus far.

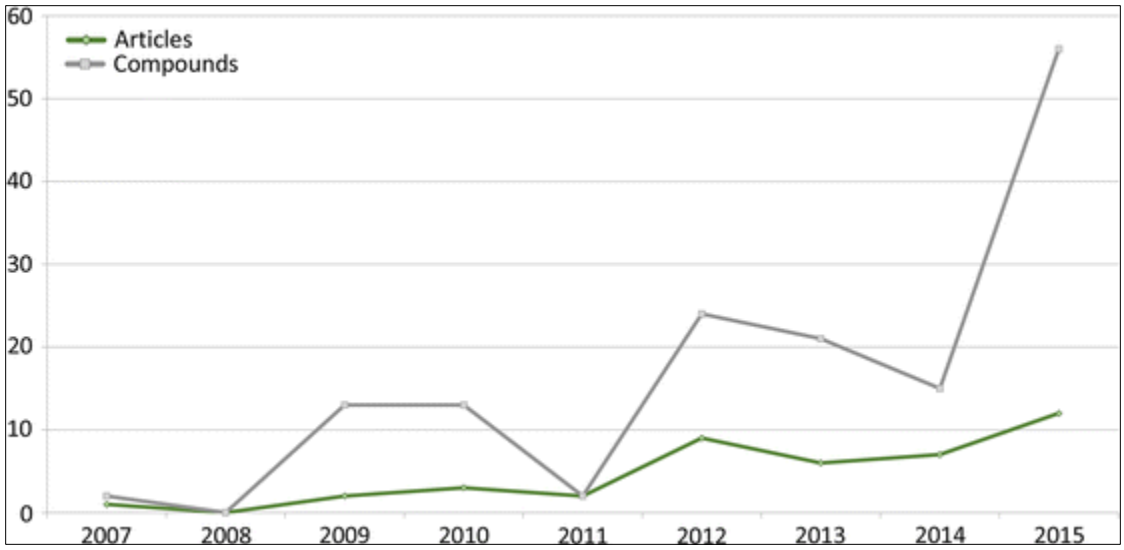


Fig. 1 Number of published articles as well as new isolated natural products from endolichenic fungi. *Graph* represents articles and compounds from the first reported metabolites in 2007 through December 2015

Biological survey of endolichenic fungi

Distribution and biodiversity

Surveys to isolate endolichenic fungi are over a decade old (Girlanda et al. 1997; Petrini et al. 1990; Suryanarayanan et al. 2005), although studies focused on isolation of secondary metabolites are more recent (see below). The first study to isolate an endolichenic fungus was undertaken in 1990 by Petrini et al. (1990), where filamentous fungi were isolated from sterilized segments of fruticose lichens belonging to genera *Cladonia* as well as *Stereocaulon.*The authors did not use chemical sterilization of lichen thallus, due to the spongy nature of the lichens, instead removing superficial contaminants from the lichen thallus with sterile tap water and sieve filters to achieve surface sterilization. Subsequently, segments of the lichen thallus were plated on 2 % malt extract, 0.4 % yeast-extract, 2 % agar, amended with 50 mg L^{-1}chlortetracycline and 1 mg L^{-1} cyclosporin (Petrini et al. 1990). This study demonstrated that lichen thalli could harbor a rich diversity of filamentous fungi belonging to the Pezizomycotina, Ascomycota. A total of 506 fungal strain types were isolated; 166 of them were isolated more than once. Girlanda et al. (1997) used two foliose lichens (*Parmelia taractica* and *Peltigera praetexta*) to study the range of fungal assemblages present. The authors used four different surface sterilization techniques to isolate fungi associated with the lichens and obtained a total of 117 fungal isolates along belonging to the Pezizomycotina, Ascomycota (Girlanda et al. 1997). Suryanarayanan et al. (2005) investigated five corticolous lichens (four foliose, and one fruticose) for non-obligate microfungi residing inside the lichen thalli in India, and also used four different sterilization procedures. In addition to isolating endolichenic fungi, Suryanarayana et al. also sought to understand whether endolichenic fungi were similar to those occurring as endophytes within the bark (termed as phellophytes) and leaves of the trees; hosts on which the selected lichens were investigated. However, there was little observed overlap between endolichenic fungi and fungal endophytes of the host tissue (Suryanarayanan et al. 2005). Li et al. (2007) surveyed endolichenic fungi from five families of lichens in the Baihua mountain of Beijing, China. The authors reported 32 taxa from 488 segments of lichen thalli, with low similarity among the lichens studied; most of the endolichenic fungi belonged to the phylum Ascomycota. Tripathi and Joshi (2015) investigated the endolichenic fungi from 14 lichen species collected in the Himalayan region of India. The authors isolated 25 cultivable isolates using culture morphology; the isolates mostly belonged to Ascomycota, but also to the Basidiomycota, and Mucoromycotina/Zygomycetes (basal fungal lineages). More recently, U'Ren et al. (2010) investigated communities of endophytic fungi in mosses and endolichenic fungi in lichens using ITS rDNA sequences obtained from cultivable fungi. The authors sought to investigate whether endolichenic fungi represent distinct ecological guilds or if they can be defined as a single group of flexible symbiotrophs capable of colonizing plants or lichens indiscriminately. Endolichenic fungal assemblages differed as a function of lichen taxonomy, rather than substrate, growth form, or photobiont. The authors found no evidence that endolichenic fungi are saprobic fungi that have been inveigled by lichen thalli; rather, their study revealed the distinctiveness of endolichenic fungal communities relative to those in living and dead plant tissues. With one notable exception, the endolichenic fungi were similar to endophytic fungi occurring in mosses (U'Ren et al. 2010, 2012). The aforementioned studies suggest that like fungal endophytes of

plants, endolichenic fungi are present in virtually all lichen species that have been examined to date, and represent a vital yet poorly studied characteristic of lichenology.

Origins and evolution

Similar to endophytic fungi, the evolutionary origins of endolichenic fungi are not well understood. Arnold et al. (2009), in a recent study on phylogenetic estimation of trophic transitions of Ascomycetes, provided some interesting insights into the origins of endophytism and the evolution of endolichenic fungi. Using ancestral state reconstruction methods, which took into account phylogenetic uncertainty; they showed that endolichenic fungi or endolichenism played a key role in evolution of endophytism within the most species rich-phylum, Ascomycota. The results of the Arnold et al. (2009) study suggest that endolichenic fungi represent a rapid evolutionary rate for fungal transitions to endophytic associations in plants. This study was also the first to document the localization of endolichenic fungi inside the lichen thalli; the authors reported that endolichenic fungi were rarely isolated from the mycobiont, but were rather preferentially associated with the green algal photobiont of the lichen thallus. The association of endolichenic fungi with an algal photobiont underscores the ecological and evolutionary similarity of endolichenic fungi with endophytic fungi, which occur in every major lineage of land plants that have been examined up to the present time (Arnold 2007; Stone et al. 2000).

Taxonomic affiliations and community structure

Endolichenic fungi are taxonomically and ecologically distinct from both lichenicolous fungi as well as the about 13,500 species of mycobiont fungi that form the lichen thalli (Arnold et al. 2009; Lutzoni and Miadlikowska 2009; Lutzoni et al. 2001). Most species isolated, as endolichenic fungi, are representatives of the Pezizomycotina, with most taxa phylogenetically related to seven orders (Fig. 2) within the subclass Eurotiomycetes, Dothideomycetes, Leotiomycetes, Pezizomycetes, and Sordariomycetes (Arnold et al. 2009; U'Ren et al. 2010). A recent paper found endolichenic fungi were phylogenetically related to a recently described novel order of fungi, Phaeomoniellales within the Eurotiomycetes (Chen et al. 2015). U'Ren et al. utilized multigene phylogenetic analysis and reported that several isolates of endolichenic fungi and fungal endophytes obtained from the continental US might represent novel species within the Xylariaceae, which require additional study. The authors also concluded that both symbiotrophic and saprotrophic fungi reside within the Xylariaceae, which is one of the largest and most diverse families within the Pezizomycotina, Ascomycota (U'Ren et al. 2016). Results from studies that have investigated endolichenic fungi suggests that the taxonomic composition, incidence of occurrence, and diversity are a consequence of the interplay of climatic patterns, geographic separation, host type, and host lineage (U'Ren et al. 2012).

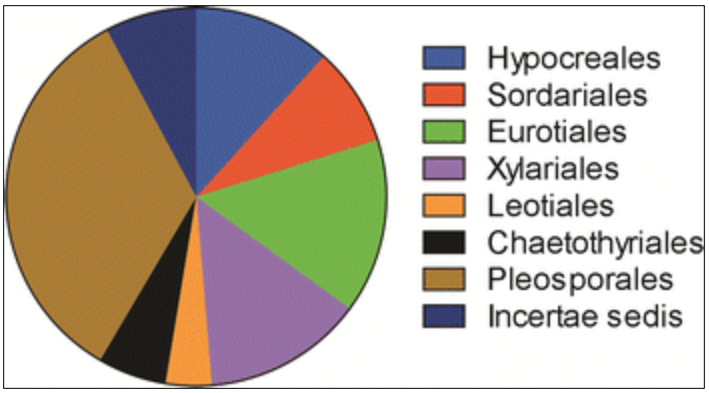


Fig. 2 Phylogenetic distribution (ordinal) of endolichenic fungi, which have been screened for bioactive secondary metabolites. Pleosporales was the dominant order with 34 % followed by Xylariales, and Hypocreales with 14, and 12 % respectively

A recent study by Chagnon et al. (2016) investigated the network construction that is responsible for organization of community structure within symbiotic fungi such as endophytic and endolichenic fungi. The authors found that endophytic fungi were more flexible, and less nested and connected compared to the endolichenic fungi; meaning plant hosts were more selective of their fungal partners (endophytes) than the lichens that harbor endolichenic fungi. In addition, it was noted that endolichenic fungi are host generalists with respect to the lichens in which they occur, but phylogenetically they are most closely related to the endophytic fungi compared to the saprobic fungi (Chagnon et al. 2016; U'Ren et al. 2010).

Isolation methods

The methods of isolating endolichenic fungi are very similar to those of endophytic fungi. Briefly, according to U'Ren et al. (2012), lichen material is transported to the laboratory and processed within 24–48 h of sampling. Samples are washed thoroughly in running tap water for 30 s. Lichen thalli are cut into small pieces, surface-sterilized, and then cut under sterile conditions into 2 mm segments. Segments are surface-sterilized by agitating sequentially in 95 % ethanol for 30 s, 10 % bleach (0.5 % NaOCl) for 2 min, and 70 % ethanol for 2 min, and surface-dried under sterile conditions (Arnold and Lutzoni 2007). After surface sterilization, the segments are placed on 2 % malt extract agar (MEA) in Petri dishes with Parafilm and incubated under ambient light/dark condition at room temperature (ca. 21.5 °C) for up to 1 year (U'Ren et al. 2010, 2012). MEA needs to be amended with antibiotics, such as Penicillin G and Steptomycin sulphate (500 mg/l), to avoid isolation of bacteria (see Stone et al. 2004 for a list of antibiotics). Emergent fungi are then isolated into pure culture (U'Ren et al. 2012). Arnold et al. (2009) utilized four different types of surface sterilization procedures for endolichenic fungi. In general, the longer lichen segments were sterilized in 0.5 % NaOCl, the fewer endolichenic fungi were recovered (Arnold et al. 2009). Additional methods of surface sterilization methods employed for endolichenic fungi have been outlined previously (Girlanda et al. 1997; Li et al. 2007; Petrini et al. 1990; Suryanarayanan et al. 2005).

Physiological and ecological roles

The biological roles of endolichenic fungi remain to be explored, but it is hypothesized that endolichenic fungi colonize the internal tissue of lichen thalli, specifically the photobiont and get nourishment and shelter from the host. In return, they may confer a multitude of benefits to their lichen host by producing a suite of biological active functional secondary metabolites.

Structural diversity and biological activities

During the past decade over 30 endolichenic microorganisms have been cultured and subjected to detailed investigations leading to the chemical characterization of over 140 new natural product structures, many of which have been shown to have a variety of biological activities (Table 1). These metabolites span a diverse array of structural types, which are outlined below.

Table 1. Bioactivity of isolated endolichenic fungal metabolites

Endolichenic fungal strain	Lichen host	Natural product(s)	Biological activity	Cell line/species strain	References
Aspergillus sp. (No. 16-20-8-1)	*Peltigeraelisabethae*var. *mauritzii*	9-acetyldiorcinol B (**90**)	$A\beta_{42}$aggregation	–	Zhao et al. (2014)
Aspergillus versicolor	*Lobaria retigera*	8-*O*-methylversicolorin A (**6**)	Cytotoxic	PC-3/H460	Dou et al. (2014)
		8-*O*-methylversicolorin B (**7**)	Cytotoxic	PC-3/H460	
Aspergillus versicolor(125a)	*Lobaria quercizans*	Diorcinol G (**87**)	Cytotoxic	PC3/A549/A2780/MDA-MB-231/HEPG2	Zhao et al. (2014)
Chaetomium elatum (No. 63-10-3- 1)	*Everniastrum cirrhatum*	Xanthoquinodin A4 (**10**)	Cytotoxic	HL-60/SMMC-7721/A-549/MCF-7/SW480	Chen et al. (2013)
		Xanthoquinodin A5 (**11**)	Cytotoxic	HL-60/SMMC-7721/A-549/MCF-7/SW480	
		Xanthoquinodin A6 (**12**)	Cytotoxic	HL-60/SMMC-7721/A-549/MCF-7/SW480	
		Xanthoquinodin B4 (**13a**)	Cytotoxic	HL-60/SMMC-7721/A-549/MCF-7/SW480	
		Xanthoquinodin B5 (**13b**)	Cytotoxic	HL-60/SMMC-7721/A-549/MCF-7/SW480	
*Coniochaeta*sp.	*Xanthoria mandschurica*	Conioxepinol B (**76**)	Cytotoxic	HeLa	Wang et al. (2010b)
		Conioxepinol D (**78**)	Cytotoxic	A549/MDA-MB-231	
Coniochaeta sp.	n/a	Coniothiepinol A (**80**)	Antibacterial	*Enterococcus faecium*(CGMCC 1.2025)/*E. faecalis* (CGMCC 1.2535)	Wang et al. (2010a)

			Antifungal	*Fusarium oxysporum*(CGMCC 3.2830)	
		Coniothienol A (**82**)	Antibacterial	*Enterococcus faecium*(CGMCC 1.2025)/*E. faecalis* (CGMCC 1.2535)	
CR1546C	*Sticta fuliginosa*	(R)-4,6,8-Trihydroxy-3,4-dihydro-1(2H)-naphthalenone (**38**)	Antifungal	*Candida albicans* (ATCC 10231)	Kim et al. (2014)
Geopyxis aff. *M ajalis*	*Pseudevernia intensa*	Geopyxin A (**111**), acetate and diester derivatives	Cytotoxic	NCI-H460/SF-268/MCF-7/PC-3M/MDA-MB-231	Wijeratne et al. (2012)
		Geopyxin B (**112**)	Cytotoxic	NCI-H460/SF-268/MCF-7/PC-3M/MDA-MB-231	
		Geopyxin C (**113**), acetate and diester derivatives	Cytotoxic	NCI-H460/SF-268/MCF-7/PC-3M/MDA-MB-231	
Myxotrichum sp.	*Cetraria islandica*	Myxodiol A (**62**)	Antifungal	*Candida albicans*(sc5314)	Yuan et al. (2013)
		Myxotrichin A (**64**)	Cytotoxic	K562	
		Myxotrichin D (**67**)	Cytotoxic	K562	
Neurospora terricola	*Everniastrum cirrhatum*	Terricollene A (**93**)	Cytotoxic	HeLa/MCF-7	Zhang et al. (2009)
		Terricollene C (**95**)	Cytotoxic	HeLa/MCF-7	
		1-*O*-methylterricollyne (**97**)	Cytotoxic	HeLa/MCF-7	
*Nodulisporium*sp. (No. 65-17-2-1)	*Everniastrum*sp.	Nodulisporiviridin A (**122**)	$A\beta_{42}$aggregation	–	Zhao et al. (2015a)
		Nodulisporiviridin B (**123**)	$A\beta_{42}$aggregation	–	
		Nodulisporiviridin C (**124**)	$A\beta_{42}$aggregation	–	
		Nodulisporiviridin D (**125**)	$A\beta_{42}$aggregation	–	
		Nodulisporiviridin E (**126**)	$A\beta_{42}$aggregation	–	
		Nodulisporiviridin F (**127**)	$A\beta_{42}$aggregation	–	
		Nodulisporiviridin G (**128**)	$A\beta_{42}$aggregation	–	

		Nodulisporiviridin H (**129**)	$A\beta_{42}$aggregation	–	
Penicillium citrinum	*Parmotrema*sp.	5′-acetyl-3,5,7′-trimethoxy-3′H-spiro [cyclohexa [2,4]diene-1,1′-isobenzofuran]-3′,6-dione (**58**)	Antioxidant	DPPH radical scavenging	Samanthi et al. (2015)
		4-acetyl-2′-hydroxy-3′,5′,6-trimethoxy biphenyl-2-carboxylic acid (**85**)	Antioxidant	DPPH radical scavenging	
*Pestalotiopsis*sp.	*Clavaroids* sp.	Ambuic acid derivative (**20**)	Antibacterial	*Staphylococcus aureus*(ATCC 6538)	Ding et al. (2009)
*Phaeosphaeria*sp.	*Heterodermia obscurata*	Phaeosphaerin A (**27**)	Cytotoxic	PC3/DU145/LNCaP	Li et al. (2012)
Preussia africana	*Ramalina calicaris*	Preussochrome C (**69**)	Cytotoxic	A549	Zhang et al. (2012)
		Preussochrome A (**79**)	Cytotoxic	A549/HCT116	
Ulocladium sp.	*Everniastrum*sp.	7-hydroxy-3, 5-dimethyl-isochromen-1-one (**52**)	Antifungal	*Candida albicans*(sc5314)	Wang et al. (2012)
		Ophiobolin P (**117**)	antibacterial	*Bacillus subtilis*/methicillin-resistant *Staphylococcus aureus*	Wang et al. (2013b)
		Ophiobolin T (**121**)	Cytotoxic Antibacterial	HepG2 *Bacillus subtilis*/methicillin-resistant *Staphylococcus aureus*/*Bacille*Calmette–Guerin	
Xylaria sp.	*Leptogium saturninum*	cyclo(N-methyl-L-Phe-L-Val-D-Ile-L-Leu-L-Pro) (**142**)	Antifungal synergist	*Candida albicans*(sc5314)	Wu et al. (2011)

Only those reported to have bioactivity during the period covered by this review are listed

Alkaloids

Solid rice cultures of the endolichenic fungus *Chaetomium globosum* (No. 64-5-8-2), originally isolated from the lichen *Everniastrum nepalense*, yielded the novel cytochalasan alkaloid

chaetoglobosin Y (**1**), which possessed a macrocyclic ring with an isoindolone moiety (Zheng et al. 2014). Rice cultures of the endolichenic fungus *Tolypocladium cylindrosporum*, which inhabits the lichen *Lethariella zahlbruckneri* yielded a new pyridine-type alkaloid tolypyridone A (**2**), as well as three novel tetramic acid derivatives [tolypocladenols A_1, A_2, and B **(3**–**5**)] (Fig. 3) (Li et al. 2015b). However, (**1**)–(**5**) did not evidence any noticeable in vitro cytotoxicity against multiple cancer cell lines (Li et al. 2015b; Zheng et al. 2014).

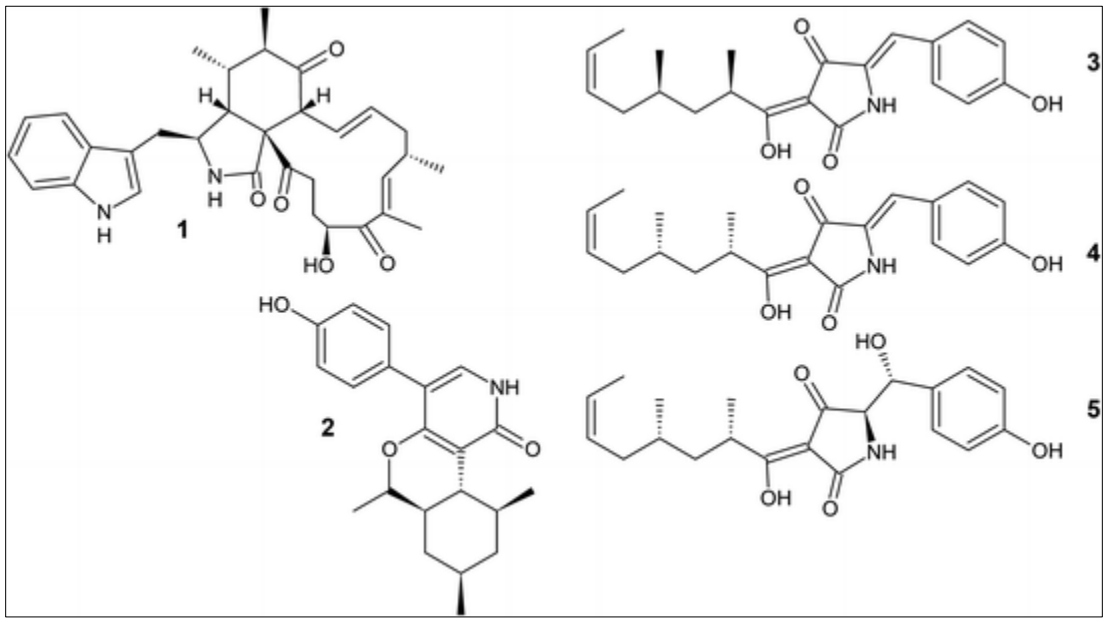
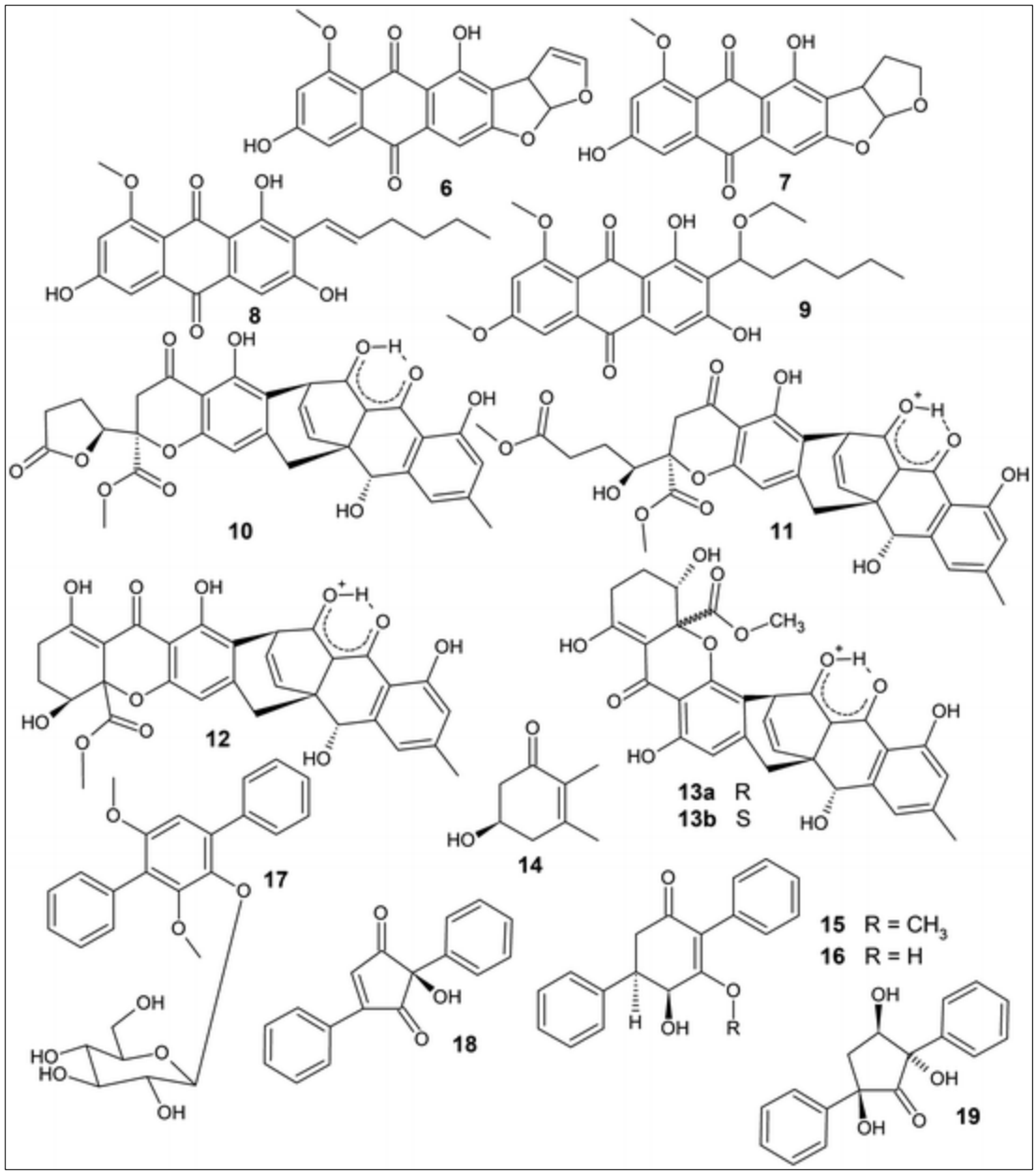


Fig. 3 Structures of new alkaloid compounds isolated from endolichenic fungi

Quinones

Anthraquinones

Investigations into the endolichenic fungal strain *Aspergillus versicolor* yielded multiple novel anthraquinone derivatives (Fig. 4). Featuring a di-furan moiety similar to versicolorin B, 8-*O*-methylversicolorin A (**6**) and 8-*O*-methylversicolorin B (**7**) were isolated, along with the alkylated anthraquinone 8-*O*-methylaverythin **(8**) (the methoxy artifact 1′-*O*-ethyl-6,8-di-*O*-methylaverantin (**9**) was also isolated) (Fig. 4) (Dou et al. 2014). The proliferation inhibition of cancer cell lines PC-3 and H460 was evaluated against (**6**)–(**9**). Moderate cytotoxic activity was evidenced by (**6**) and (**7**), with IC_{50} values of 12.6 and 19.5 μM against PC-3 cells and 17.3 and 27.2 μM against H460 cells, respectively (Dou et al. 2014).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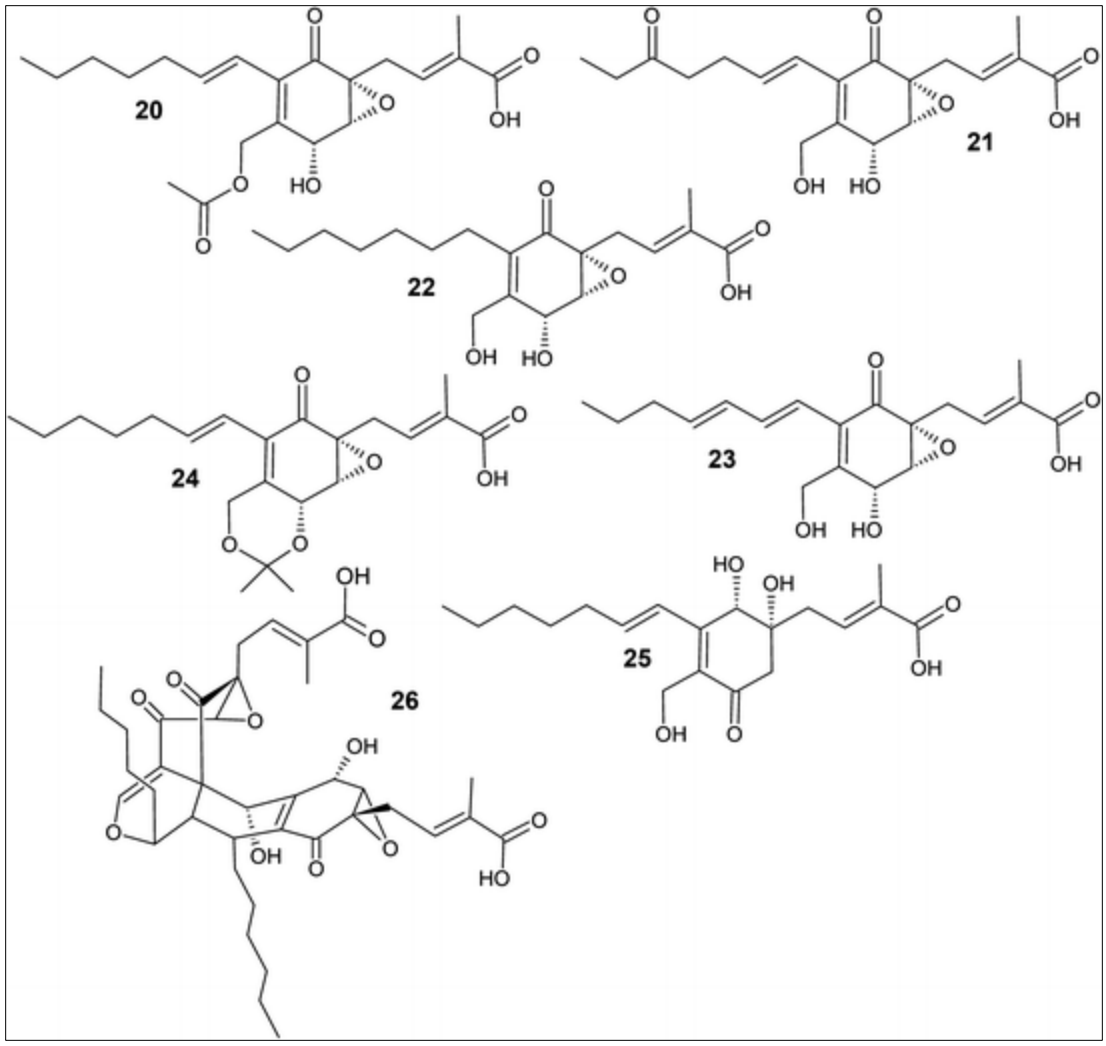
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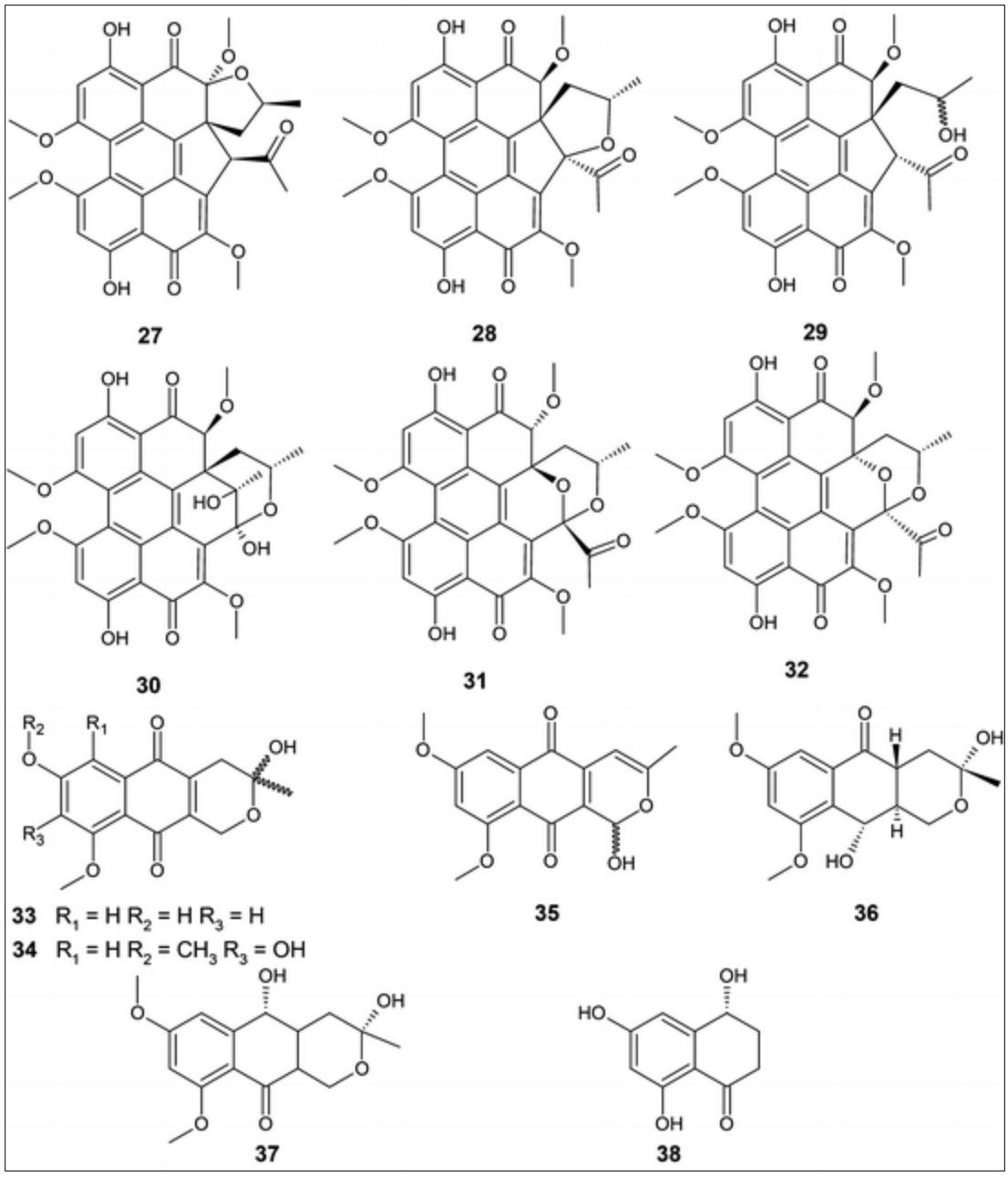


Fig. 4 Structures of new quinone compounds isolated from endolichenic fungi

The xanthoquinodins represent an unusual xanthone-anthraquinone heterdimeric skeleton that derives from two polyketide metabolites. The first polyketide forms an anthraquinone monomer via decarboxylation of the terminal chain portion, and the second a xanthone monomer by decarboxylation/oxidization reactions, which is then fused to the anthoquinone monomer in several different configurations (Tabata et al. 1993). Investigation into the endolichenic fungus *Chaetomium elatum* (No. 63-10-3-1) revealed five novel xanthoquinodins, A4–A6 (**10**)–(**12**), B4 (**13a**) and B5 (**13b**) (Fig. 4) (Chen et al. 2013). All five compounds displayed cytotoxic activity against five cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480), with

(**12**) possessing low-μM activities against all cell lines (IC_{50} ranging from 2 to 6 μM) (Chen et al. 2013).

Quinones

From the culture of the endolichenic fungus Pleosporales sp., six new quinone metabolites were isolated. The cyclohexenone (5R)-5-hydroxy-2,3-dimethylcyclohex-2-en-1-one (**14**) was identified, along with **t**hree terphenyl derivatives, cucurbitarins A (**15**) and B (**16**), as well as the glycosylated cucurbitarin C (**17**). Two related cucurbitarins with a cyclopentenyl core were also isolated, cucurbitarins D (**18**) and E (**19**) (Fig. 4) (Jiao et al. 2015).

Ambuic acid is a highly functionalized cyclohexenone, initially isolated from rainforest endophytic fungi *Pestalotiopsis* sp. and *Monochaetia* sp. (Li et al. 2001). Ambuic acid initially evidenced antifungal activity (Li et al. 2001), but also has moderate antibacterial activity and quorum-sensing inhibitory activity (Nakayama et al. 2009). Six novel derivatives of the highly bioactive ambuic acid were isolated from the crude extract of the endolichenic fungus *Pestalotiopsis* sp. inhabiting the lichen *Clavaroids* sp. (Ding et al. 2009). Compounds (**20**)–(**25**) displayed varying levels of oxidation and stereochemistry around an ambuic acid skeleton. The dimeric quinone (**26**), similar to torreyanic acid, was isolated from the same fungal strain (Fig. 4). Metabolites (**20**)–(**26**) were screened for antibacterial activity against a panel of Gram-positive and Gram-negative bacterial strains. Only (**20**) exhibited moderate inhibition of *Staphylococcus aureus* (IC_{50} of 27.8 μM, compared to 43.9 μM for ambuic acid), and none of these compounds demonstrated antifungal activity against *Aspergillus fumigatus* (Ding et al. 2009).

Few perylenequinonoid pigments have been discovered, and those originate mostly from Ascomycete fungi (Zhou and Liu 2010). These pigments are attractive cytotoxic metabolites, as they are transformed to excited triplet states by absorption of light energy, which can react with oxygen to generate reactive oxygen species that disrupt protein kinase C activity in mammalian cells (Morgan et al. 2009). From the endolichenic fungus *Phaeosphaeria* sp., occurring in the lichen *Heterdermia obscurata,* six novel perylenequinones possessing an unusual α,β-unsaturated ketone moiety were isolated, phaeosphaerins A–F (**27**–**32**) (Fig. 4) (Li et al. 2012). Compounds (**27**)–(**32**) were evaluated for cytotoxicity against PC3, DU145, and LNCaP cancer cell lines, with (**27**) demonstrating growth inhibitions of PC3 (IC_{50} 5.84 μM), DU145 (IC_{50}10.77 μM), and LNCaP (IC_{50} 10.76 μM). Further investigations revealed that (**27**) accumulated in the lysosomes of tumor cells, and its inhibitory activity was potentiated using light irradiation (Li et al. 2012).

Three new herbarin-derived adducts—7-desmethylherbarin (**33**), 8-hydroxyherbarin (**34**), and 1-hydroxydehydroherbarin (**35**)—were isolated from the endolichenic fungal strain *Corynespora* sp. BA-10763, which occurs in the cavern beard lichen *Usnea cavernosa* (Fig. 4) (Paranagama et al. 2007; Wijeratne et al. 2010). The biosynthetically related compounds corynesporol (**36**) (Paranagama et al. 2007) and 9-*O*-methylscytalol A (**37**) (Wijeratne et al. 2010) were also isolated. These pyranonapthoquinones were evaluated for their migration inhibitory activities of PC-3M and MDA-MD-231 cancer cell

lines; none of the isolated metabolites possessed significant inhibitory activity (Paranagama et al. 2007; Wijeratne et al. 2010).

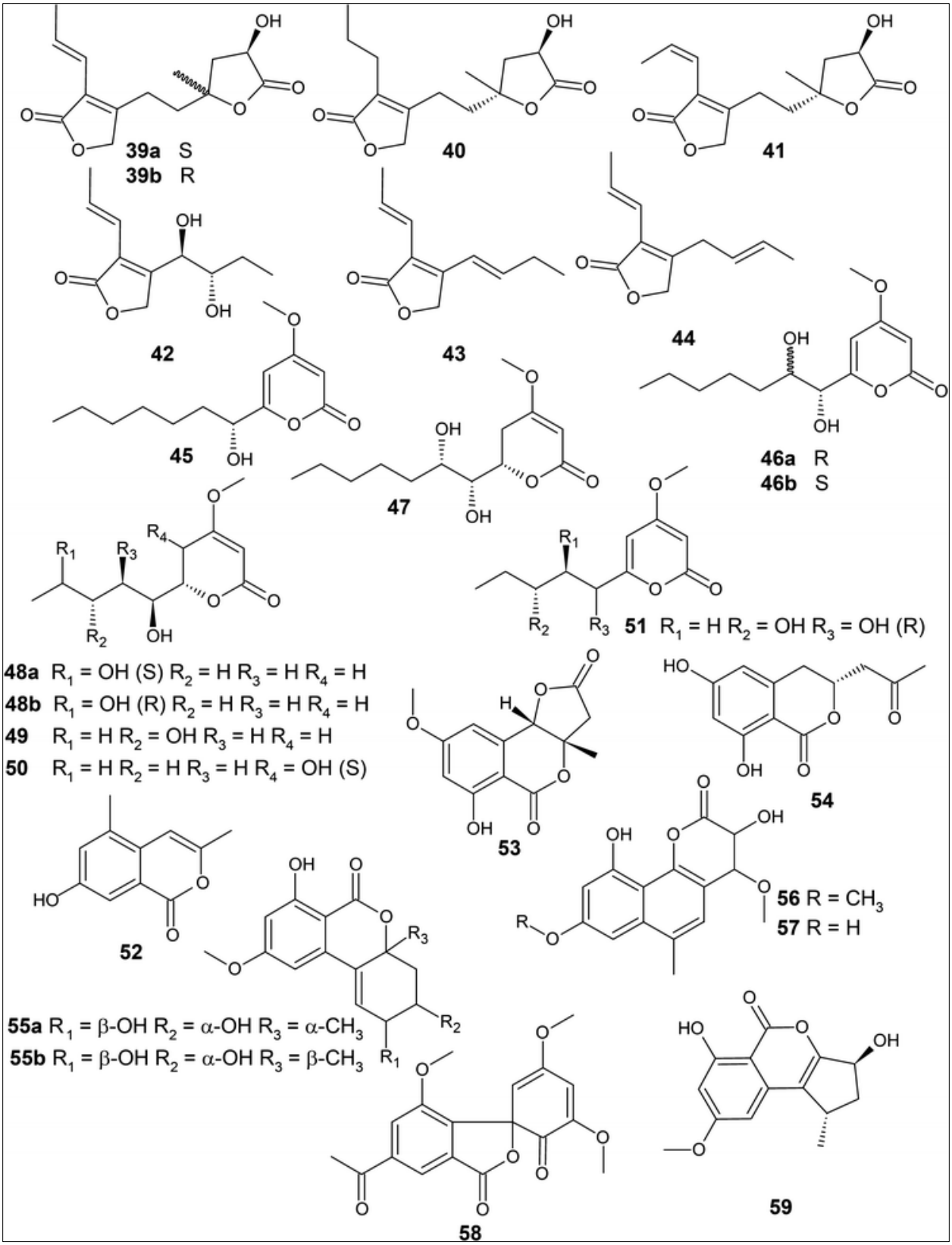
Other quinone derivatives

A new napthalone ((R)-4,6,8-trihydroxy-3,4-dihydro-1(2H)-naphthalenone (**38**)) was obtained from a Costa Rican endolichenic fungus *Xylariaceae* sp. CR1546C from the lichen *Sticta fuliginosa* (Kim et al. 2014). The napthalone demonstrated weak antimicrobial activity against *Bacillus subtilis* (MIC 150 μg mL^{-1}) and *Candida albicans* (MIC 100 μg mL^{-1}) (Kim et al. 2014).

Oxygen heterocycles

Furanones

Seven novel furanone metabolites were isolated from the crude extract of the endolichenic fungus *Peziza* sp. inhabiting the lichen *Xanthoparmelia* sp. (Zhang et al. 2014), the pezizolides A–G (**39**)–(**44**). Compounds (**39**)–(**41**) contained a bis-furanone moiety, while (**42**)–(**44**) possessed only a single furanone ring (Fig. 5). The compounds were all tested for cytotoxicity against HeLa, A549, MCF-7, HCT116, and T24 cancer cell lines, and for potential antimicrobial activity against *B. subtilis*, *S. aureus*, and *C. albicans*; however, (**39**)–(**44**) did not show any detectable activity at the 20 μg mL^{-1} level (Zhang et al. 2014).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46b S
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48b R1 = OH (R) R2 = H R3 = H R4 = H
49 R1 = H R2 = OH R3 = H R4 = H
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55a R1 = β-OH R2 = α-OH R3 = α-CH3
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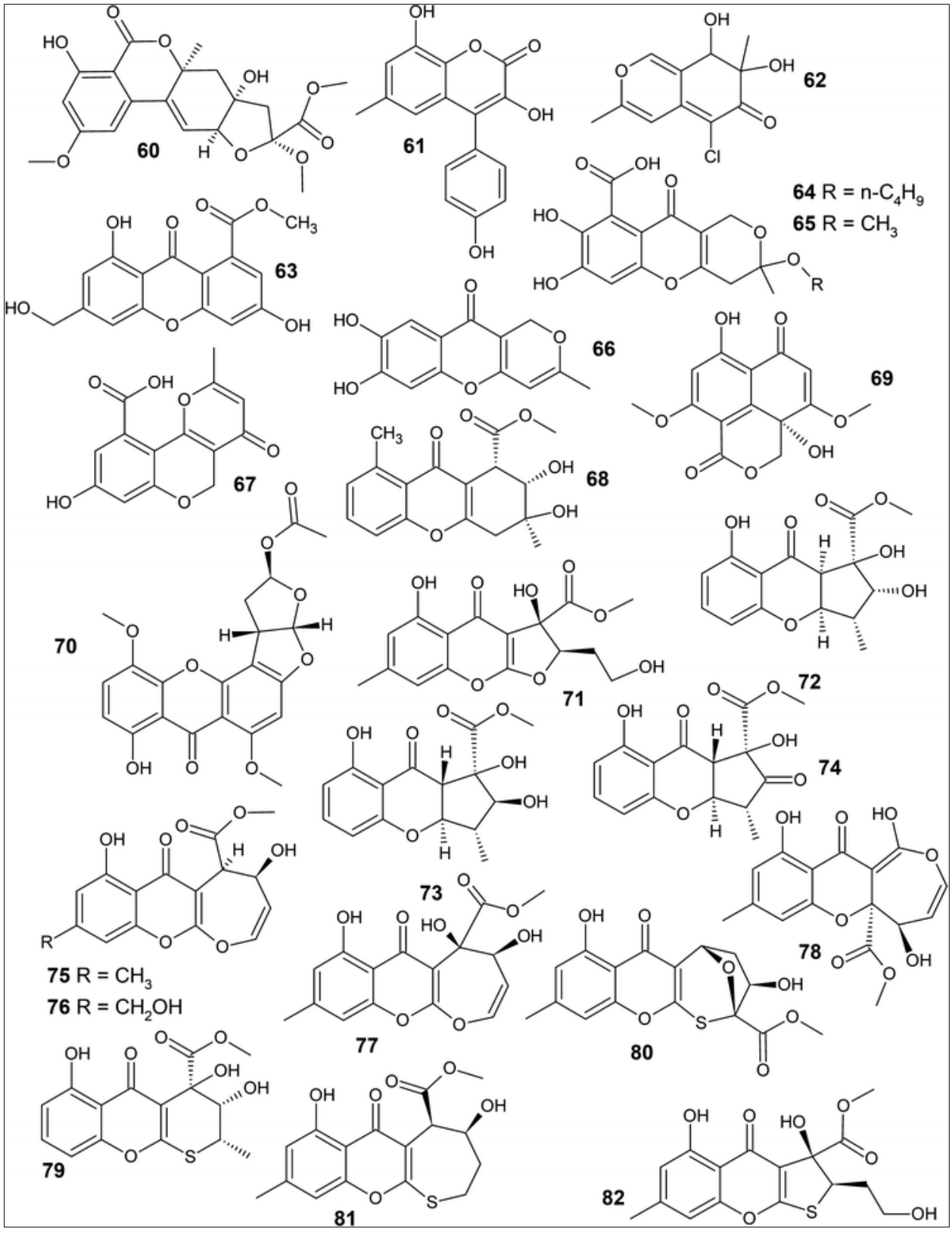


Fig. 5 Structures of new oxygen-containing herterocyclic compounds isolated from endolichenic fungi

Pyrones

The new α-pyrone derivatives nodulisporipyrones A–D (**45**)–(**47**) were isolated from a rice culture of the fungal strain *Nodulisporium* sp. (65-12-7-1), symbiotic with the lichen *Everniastrum* sp.(Zhao et al. 2015b), and the novel derivatives necpyrone A, B, D, and E [(**48**)–(**50**), respectively)] have been detected in the endolichenic fungus *Nectria* sp., occurring in the lichen *Pamelia* sp. (Figure 5) (Li et al. 2015a). A dehydrogenated structure, the new metabolite necpyrone C (**51**), was also isolated from *Nectria* sp. (Li et al. 2015a). The α-pyrones (**45**)–(**47**) failed to demonstrate any activity in in vitro antibacterial assays against *S. aureus* or *E. coli* (MIC > 1000 μg mL^{-1}), and weak activity against the fungi *Aspergillus niger* and *C. albicans* (MIC 31 and 250 μg mL^{-1}, respectively) ((Zhao et al. 2015b). And a study against six human cancer cell lines (K562, MDA-MB-231, MCF-7, SW620, HT29, and HeLa) showed no cytotoxicity (IC_{50} > 120 μM) for compounds (**48**)–(**51**) (Li et al. 2015a).

Benzopyranoids

Endolichenic fungi have yielded a variety of benzypyranoid and coumarin derivatives, with varying bioactivity. Two novel polyketides, 7-hydroxy-3, 5-dimethyl-isochromen-1-one (**52**) and 6-hydroxy-8-methoxy-3a-methyl-3a,9b-dihydro-3H-furo[3,2-c]isochromene-2,5-dione (**53**), were isolated from the endolichenic fungus *Ulocladium* sp. and (**53**) possessed a completely novel tricyclic skeleton as part of its structure (Fig. 5) (Wang et al. 2012). The coumarin derivative 6,8-dihydroxy-(3R)-(2-oxopropyl)-3,4-dihydroisocoumarin (**54**) was obtained from the Costa Rican endolichenic fungus coded CR1546C (Fig. 5) (Kim et al. 2014). The polyketide 7-hydroxy-3, 5-dimethyl-isochromen-1-one (**52**) evidenced mild antifungal activity against *Candida albicans* SC 5314, possessing an IC_{50} of 97.9 ± 1.1 μM (Wang et al. 2012).

The ethyl acetate extracts of the endolichenic fungi *Nigrospora sphaerica* (No. 83-1-1-2, found in *Parmelinella wallichiana*), *Alternaria alternata* (No. 58-8-4-1, from the lichen *Usnea aciculifera*) and *Phialophora* sp. (No. 96-1-8-1, from *Cetrelia braunsiana*) yielded the diastereomeric pair (+)-(2S,3S,4aS)-altenuene (**55a**) and (−)-(2S,3S,4aR)-isoaltenuene (**55b**) (Fig. 5) (He et al. 2012). From a suite of eight novel metabolites from the fungus *Pleosporales*sp., two benzocoumarins were isolated: 3,10-dihydroxy-4,8-dimethoxy-6-methylbenzocoumarin (**56**) and 3,8,10-trihydroxy-4-methoxy-6-methylbenzocoumarin (**57**),(Jiao et al. 2015) and 5′-acetyl-3,5,7′-trimethoxy-3′H-spiro [cyclohexa [2,4]diene-1,1′-isobenzofuran]-3′,6-dione (**58**) was obtained from *Penicillium citrinum*, an endolichenic fungal strain from a Sri Lankan *Parmotrema* species (Samanthi et al. 2015). The coumarin 3,8-dihydroxy-4-(4-hydroxyphenyl)-6-methylcoumarin (**61**) was obtained from the endolichenic fungus *Tolypocladium cylindrosporum*, inhabiting the lichen *Lethariella zahlbruckneri* (Fig. 5) (Li et al. 2015b). Of the benzocoumarins from endolichenic fungi, (**58**) demonstrated moderate antioxidant activity, scavenging the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with an IC_{50}value of 159.7 ± 22.3 μg mL^{-1}.

Altenusin derivatives originate from polyketide biosynthesis pathways, generally containing seven acetate units and forming either a bicyclic or tricyclic ring skeleton, such as 6/6/6, found with altenunenes, or 6/6/5 common to the rubralactones. Two new altenusins, phialophoriol (**59**)

and xinshengin (**60**), were isolated from a *Phialophora* spp. (No. 39-1-5-1) obtained from the lichen *Cladonia ochrochlora* Flörke (Fig. 5). Xinshengin (**60**) is composed of a unique altenuene/tetrahydrofuran-fused tetracyclic skeleton, with rings of 6/6/6/5 (Ye et al. 2013).

Myxidiol A (**62**), a novel austdiol analog, was isolated from the endolichenic fungus *Myxotrichum* sp. on the lichen *Cetraria islandica* (Fig. 5). This compound is remarkable in that it is the first endolichenic fungal metabolite isolated containing a halogen, a chlorine atom located at position five. Myxidiol A (**62**) demonstrated showed minimal antifungal activity against *Candida albicans* (sc5314) with a MIC of 128 μg mL^{-1} (Yuan et al. 2013).

Xanthones

Endolichenic fungi have demonstrated a great deal of biosynthetic plasticity in the production of xanthones and xanthone derivatives. The xanthone conioxanthone A (**63**) was isolated from a culture of *Coniochaeta* sp. originating on the lichen *Xanthoria mandschurica* (Fig. 5) (Wang et al. 2010b). A *Myxotrichum* sp. living in the lichen *Cetraria islandica* yielded three related fulvic acid derivatives myxotrichin A–C (**64**)–(**66**), and a citromycetin analog myxotrichin D (**67**) (Fig. 5). Of the four xanthone derivatives from *Myxotrichum* sp., (**64**) and (**67**) demonstrated weak in vitro cytotoxic activity against the K562 cell line, with IC_{50} values 32 and 20 μM, respectively (Yuan et al. 2013). The xanthone derivative preussochromone B (**68**) was isolated from *Preussia africana* (living in the lichen *Ramalina calicaris*), which also yielded preussochromone C (**69**), with a corymbiferone skeleton (Fig. 5). Compound (**69**) was highly cytotoxic against A549 cells, with an IC_{50} of 5.8 μM, yet evidenced no activity (IC_{50} > 20 μM) against MCF-7, HeLa, and HCT116 cell lines (Zhang et al. 2012). Finally, oxisterigmatocystin D (**70**) was isolated from *Aspergillus* sp. (No. 16-20-8-1) associated with the lichen *Peltigeraelisabethae* (Zhao et al. 2014).

Coniofurol A (**71**) was identified as a new member of the furochromenone class of oxygen heterocycles, possessing the representative ring-contracted xanthone structure with novel substituents on the aryl and furan rings (Fig. 5) (Wang et al. 2010b). Preussochromes D–F (**72**)–(**74**) were similarly ring-contracted xanthones from solid cultures of an endolichenic fungus *Preussia africana* (Fig. 5) (Zhang et al. 2012). And novel ring-expanded xanthones were discovered from *Coniochaeta* sp. Conioxepinols A–D (**75**)–(**78**) possessed the base oxepinochromenone skeleton, with differing configurations at C7 and C8 (for conioxepinol A–C) and C3 and C6 (for conioxepinol D) and altered substitution patterns on the aryl and oxepine rings (Fig. 5) (Wang et al. 2010b). Conioxepinol B (**76**) showed modest cytotoxicity activity against HeLa cell line, with an IC_{50} value of 36 μM, while conioxepinol D (**78**) demonstrated cytotoxcity against A549 and MDA-MB-231 cells, possessing IC_{50} values of 40 and 41 μM, respectively (Wang et al. 2010b).

Sulfur-containing chromenones

Several relatively rare sulfur-containing chromenone structures have been isolated from endolichenic fungal cultures. Preussochromone A (**79**), isolated from the fungus *Preussia africana*, was found to possess a 3,4-dihydrothiopyrano[2,3-b]chromen-5(2H)-one structure, which had not previously been detected in a naturally-occurring product, only as a synthetic

derivative (Majumdar and Jana 2001; Palmisano et al. 2007; Zhang et al. 2012). Preussochromone A (**79**) demonstrated significant cytotoxic activity against A549 and HCT116 cell lines, with IC_{50} values of 8 and 11 μM, respectively (Zhang et al. 2012). Three ring-altered, sulfur-contaning xanthone derivatives were observed in cultures of the endolichenic fungus *Coniochaeta* sp. (Figure 5). Coniothiepinols A (**80**) and B (**81**) are the first recorded naturally-occurring thiepinols, containing the unique 4,5-dihydro-2H-thiepino[2,3-b]chromen-6(3H)-one skeleton. Coniothiepinol A (**80**) has a distinctive C5–C8 ether linkage, resulting in a 8-oxa-2-thia-bicyclo[3.2.1]octane partial structure. Coniothienol A (**82**) possessed a ring-contracted 2H-thieno[2,3-b]chromen-4(3H)-one base structure (Wang et al. 2010a). When evaluated against *Enterococcus faecium* (CGMCC 1.2025) and *E. faecalis* (CGMCC 1.2535), (**82**) demonstrated significant activity against the two Gram-positive bacterial strains (IC_{50} values of 2.00 and 4.89 μg mL^{-1}, respectively), and (**80**) showed modest inhibition against the Gram-positive strains (3.93 and 11.51 μg mL^{-1}, respectively) as well as the plant pathogen *Fusarium oxysporum* (CGMCC 3.2830) with an IC_{50} of 13.12 μg mL^{-1} (Wang et al. 2010a).

Simple aromatic compounds

Simple aromatic structures, or aromatic rings bound together by carbon–carbon or ether linkages represent a family of natural product skeletons observed in endolichenic fungi. Discovery efforts involving the endolichenic fungal strain *Scopulariopsis* sp. (occurring on *Cladonia gracilis*) led to the isolation of two new naphthalene derivatives, 1-(4′-hydroxy-3′,5′-dimethoxy-phenyl)-1,8-dimethoxynaphthalen-2(1H)-one (**83**) and 1,8-dimethoxynaphthalen-2-ol (**84**) (Fig. 6) (Yang et al. 2012). The biphenyl compound 4-acetyl-2′-hydroxy-3′,5′,6-trimethoxy biphenyl-2-carboxylic acid (**85**) was isolated from *Penicillium citrinum* (Fig. 6), an endolichenic fungal strain from a Sri Lankan lichen *Parmotrema* sp., and demonstrated radical scavenging activity in a DPPH assay with an IC_{50} value of 69.6 μg mL^{-1} (Samanthi et al. 2015).

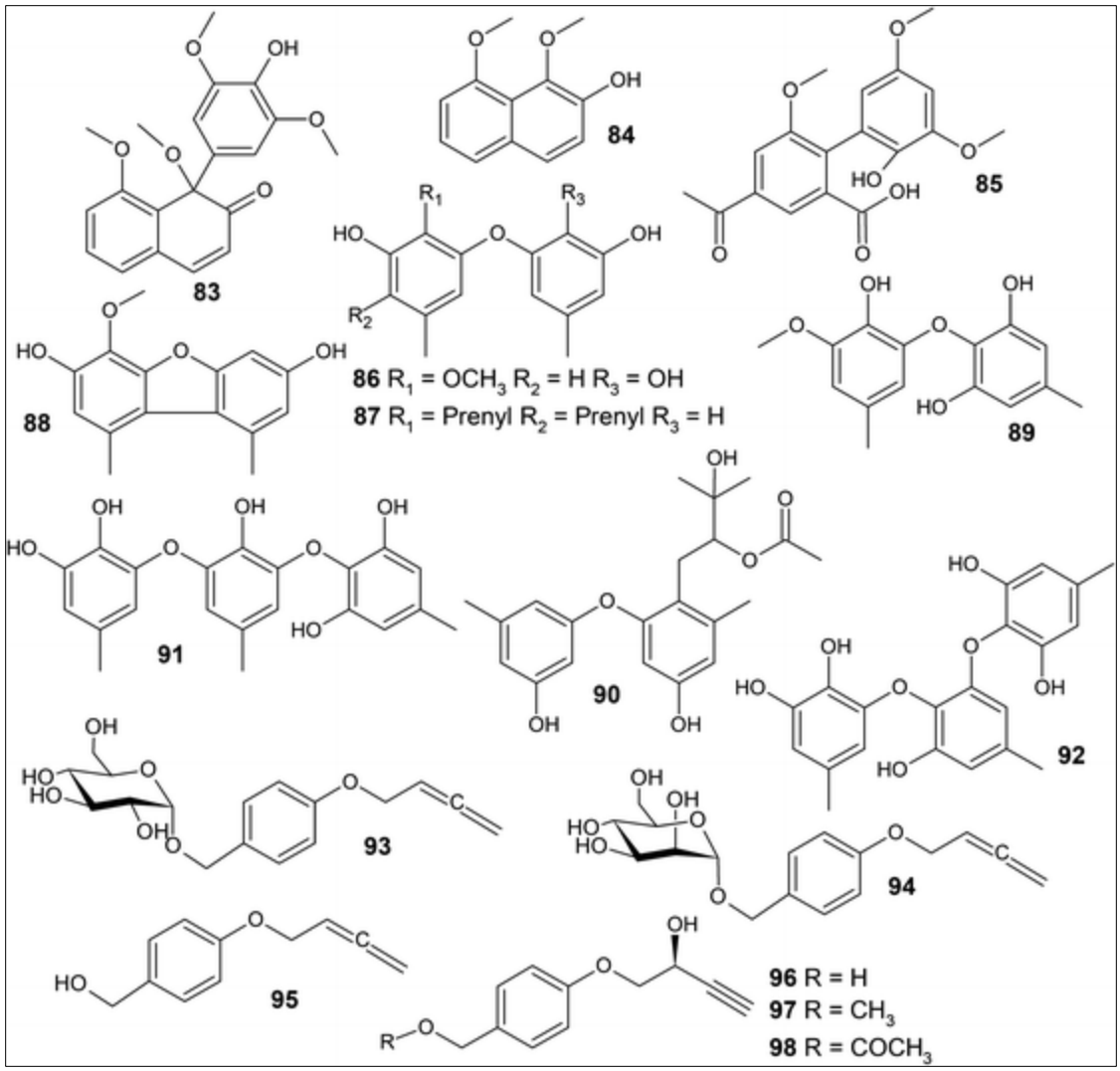


Fig. 6 Structures of new simple aromatic compounds isolated from endolichenic fungi

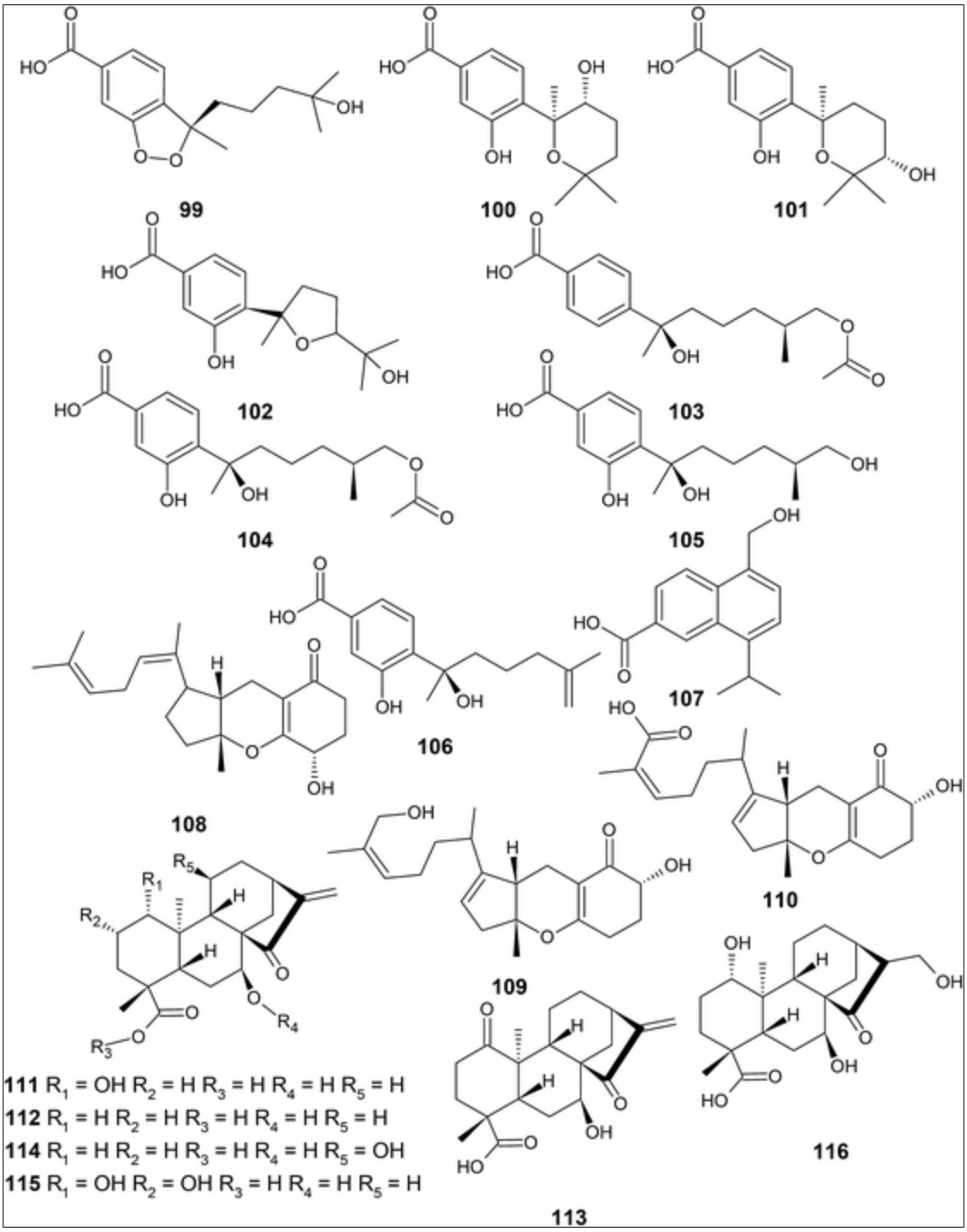
Four biphenyl ether compounds, diorcinols F–H (**86**)–(**88**) and 3-methoxyviolaceol-II (**89**), were found in the endolichenic fungus *Aspergillus versicolor* (125a) from the lichen *Lobaria quercizans* (Fig. 6) (Li et al. 2015c), and another biphenyl, 9-acetyldiorcinol B (**90**), was isolated from *Aspergillus* sp. (No. 16-20-8-1), endolichenic with *Peltigera elisabethae* var. mauritzii (Fig. 6) (Zhao et al. 2014). *Aspergillus versicolor* (125a) also yielded two new tris (pyrogallol ethers), sydowiols D (**91**) and E (**92**) (Fig. 6), which featured a triphenyl structure joined by aryl-ether bridges (Li et al. 2015c).

The simple phenyl ethers demonstrated a range of bioactivities; (**87**) exhibited moderate cytotoxicity against tested human cancer cell lines PC3, A549, A2780, MDA-MB-231, and HEPG2, with IC_{50} values ranging from 19.0 to 31.0 μM, yet was inactive against *Candida albicans* (MIC > 64 μg mL^{-1}) (Li et al. 2015c). The metabolite 9-acetyldiorcinol B (**90**) inhibited $A\beta_{42}$ aggregation at the 100 μM level (Zhao et al. 2014).

Neurospora terricola, isolated from the lichen *Everniastrum cirrhatum*, has yielded several unique allenyl and alkynyl phenyl ether structures. Terricollenes A–C (**93**)–(**95**) contained a p-(buta-2,3-dienyl ether)phenyl moiety, similar to xyloallenolide A (Fig. 6) (Lin et al. 2001) and the eucalyptenes (Arnone et al. 1993), and (**93**) and (**94**) were both glycosylated with glucose and mannose, respectively (Zhang et al. 2009). Three alkynyl phenyl ethers were also isolated, terricolyne (**96**), 1-*O*-methylterricolyne (**97**), and 1-*O*-acetylterricolyne (**98**) (Fig. 6). Compounds (**93**), (**95**), and (**97**) evidenced modest cytotoxic activity against HeLa cells, with IC_{50} values ranging from 53.3 to 92.6 μM, and (**93**) also displayed activity against MCF-7 cell line, with an IC_{50} value of 59.2 μM (Zhang et al. 2009).

Terpenes

Sesquiterpenoids constitute a broad structural class of natural products biosynthesized by a diverse range of organisms. Eight new bisabolane sesquiterpenoids, (−)-(R)-cyclohydroxysydonic acid (**99**), (−)-(7S,8R)-8-hydroxysydowic acid (**100**), (−)-(7R,10S)-10-hydroxysydowic acid (**101**), (−)-(7R,10R)-iso-10-hydroxysydowic acid (**102**), (−)-12-acetoxy-1-deoxysydonic acid (**103**), (−)-12-acetoxysydonic acid (**104**), (−)-12-hydroxysydonic acid (**105**), and (−)-(R)-11-dehydrosydonic acid (**106**), were isolated from the endolichenic fungus *Aspergillus versicolor* (125a) living in the lichen *Lobaria quercizans* (Fig. 7) (Li et al. 2015c). These compounds are formed via a series of oxidation, reduction, cyclization, and esterification reactions of the bisabolane nucleus, arising from the mevalonic acid pathway (Li et al. 2015c). The fungal strain *Periconia* sp. (No. 19-4-2-1), endolichenic with *Parmelia* sp., yielded the novel cadinane-type sesquiterpenoid Pericoterpenoid A (**107**), which demonstrated moderate antifungal potential against *Aspergillus niger* (MIC 31 μg mL^{-1}) (Wu et al. 2015). From a Czapek's culture of the endolichenic fungus *Ulocladium* sp. (cultured from *Everniastrum* sp.), novel mixed terpenoids possessing a tricyclic core, the tricycloalternarenes F–H (**108**)–(**110**), were identified (Fig. 7) (Wang et al. 2013a).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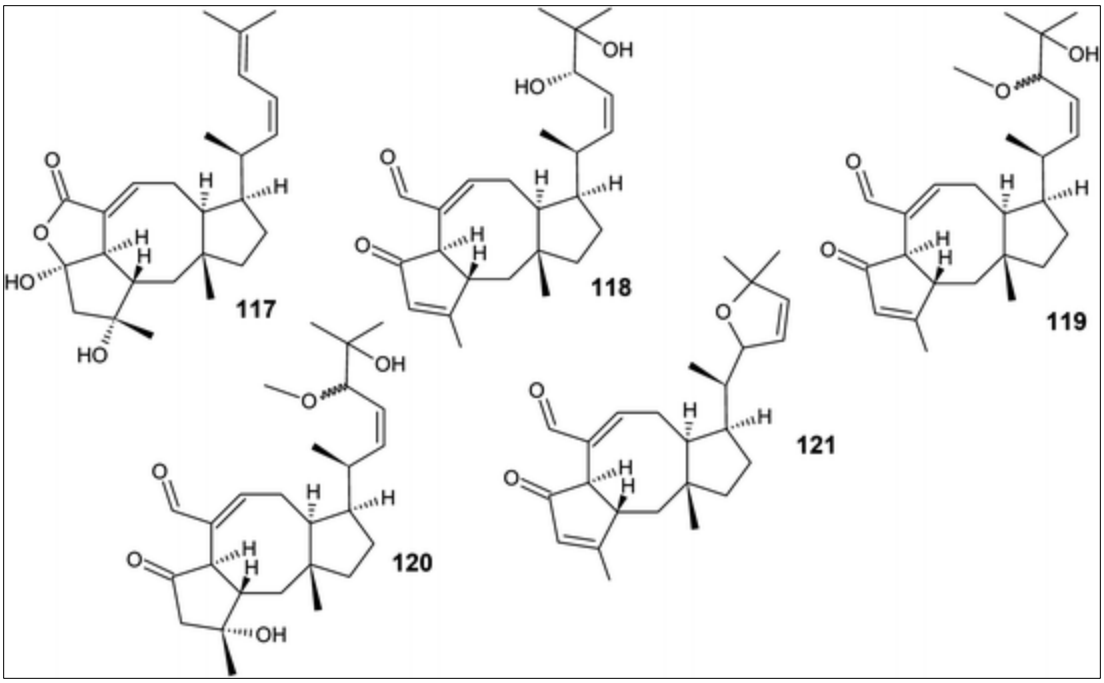

Fig. 7 Structures of new terpenoid compounds isolated from endolichenic fungi

Ent-kaurane diterpenoids of fungal origin have been rare and mainly reported from *Gibberella fujikuroi* and *Phaeosphaeria* sp. L487 (Kawaide 2006), though this class of diterpenoids has frequently been present in several plant families (Garcia et al. 2007). Obtained from the host *Pseudevernia intensa*, cultures of two endolichenic fungi (*Geopyxis* aff. *majalis*, and *Geopyxis*sp. AZ0066) led to the isolation of new ent-kaurane diterpenes, geopyxins A–F (**111**)–(**116**) (Fig. 7). Geopyxin B (**112**) was the only natural geopyxin to demonstrate cytotoxicity activity in the low micromolar range against the cancer cell lines NCI-H460, SF-268, MCF-7, PC-3M, and MDA-MB-231; however, the monoacetate and diacetate derivatives of (**111**) and the methyl esters of (**111**)–(**113**) showed low or sub micromolar activities against the cell lines, as well as activating the heat-shock response (Wijeratne et al. 2012).

Ophiobolins are a family of naturally occurring sesterterpenes characterized by a unique C_5–C_8–C_5 tricyclic ring system. Via an OSMAC (one strain, many compounds) method, wherein culture conditions are altered to prompt production of a different metabolite profile, cultures of *Ulocladium* sp. (endolichenic in *Everniastrum* sp.) grown on potato dextrose broth (PDB) produced five novel ophiobolane sesterterpenes, the ophiobolins P–T (**117**)–(**121**) (Fig. 7) (Wang et al. 2013b). Ophiobolin T (**121**) exhibited strong cytotoxic activities against HepG2 with an IC_{50} value of 0.24 μM, and (**117**) and (**121**) demonstrated moderate antibacterial activity against *Bacillus subtilis* and methicillin-resistant *Staphylococcus aureus*. Ophiobolin T (**121**) also presented moderate antibacterial activity against the *Bacille* Calmette–Guerin strain. The heightened activity of (**121**) compared to the other ophiobolins suggested that the furan ring on the side-chain at C-15 significantly influenced the bioactivity of ophiobolin sesterterpenes (Wang et al. 2013b).

Steroids

Viridins represent a fairly unique class of natural product sterol derivatives, featuring a furan ring fused to a pregnane or androstane steroid nucleus at the C-4 and C-6 position. These steroid structures have attracted interest for their potent antifungal, antibiotic, phytotoxic, and anti-inflammatory activities (Hansen 1995; Wipf and Halter 2005). Eight novel viridins, nodulisporiviridins A–H (**122**)–(**129**) (Fig. 8), were isolated from the endolichenic fungus *Nodulisporium* sp. (No. 65-17-2-1). The nodulisporiviridins have a unique skeleton, featuring a cleaved A ring at either C-1 or C-10 in the 10*R* or 10*S* configuration (Zhao et al. 2015a). All nodulisporiviridins possessed inhibitory activity in an anti-$A\beta_{42}$ aggregation assay, with nodulisporiviridin G (**128**) exhibiting the most potent anti-aggregation activity (IC_{50} of 1.2 μM), and all improved short-term memory in a human $A\beta_{42}$ transgenic AD fly model (Zhao et al. 2015a).

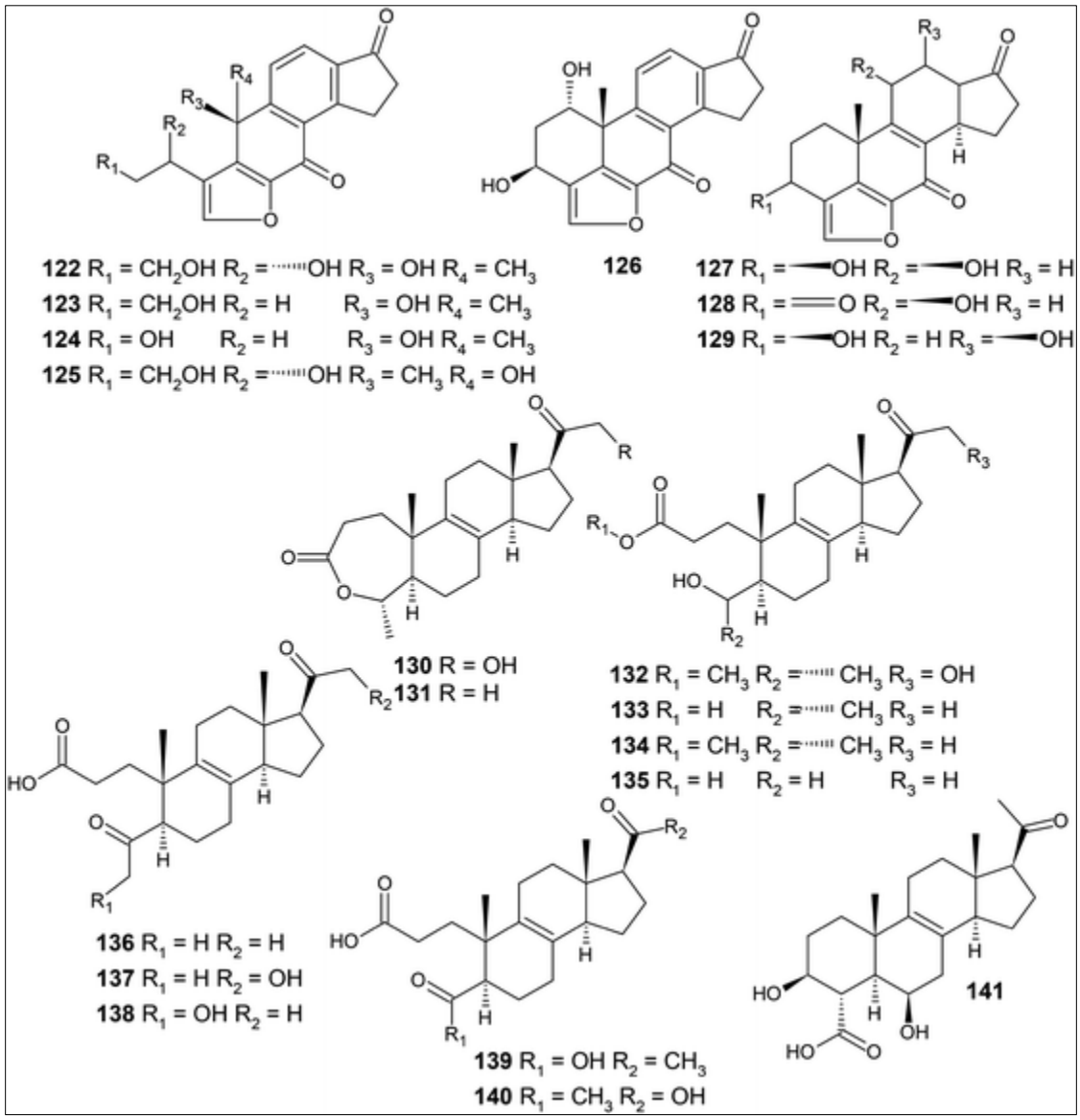

Fig. 8 Structures of new steroid compounds isolated from endolichenic fungi

Progesteroids, C21 steroid skeletons possessing a C-2 alkyl side chain at C-17, are fairly commonly found in nature; however, the 4-methyl derivatives are only rarely detected (De Rosa et al. 1999). From the endolichenic fungus *Nodulisporium* sp. (No. 65-17-2-1), the first examples of 3,4-*seco*-4-methyl-pregnan steroids were isolated, nodulisporisteroid A (**130**) and B (**132**) (Fig. 8) (Zheng et al. 2013). By employing an OSMAC method, ten additional 4-methyl-progesteroid derivatives were obtained from the same endolichenic fungus, and were termed nodulisporisteroids C (**131**) and D–L (**133**)–(**141**) (Fig. 8). However, none of the compounds demonstrated cytotoxic activity against HL-60, SMMC-7721, A-549, MCF-7, or SW480 human cancer cell lines (Zhao et al. 2015c).

Peptides

At the time of this review, only two peptides have been reported from endolichenic fungi. From the endolichenic fungus, *Xylaria* sp. (75-1-3-1), two proline-containing cyclopentapeptides. were isolated. Cyclo-(-NMePhe-Pro-Leu-Ile-Val-) (**142**) and cyclo-(-Leu-Pro-Leu-Ile-Val-) (**143**) were tested for antifungal activity against *C. albicans* (Fig. 9) (SC5314). While neither (**142**) or (**143**) evidenced antifungal activity at the highest concentration tested (100 μg mL^{-1}), (**142**) showed synergistic activity at concentrations of 6.3 μg mL^{-1} in combination with 0.004 μg mL^{-1} ketoconazole, yielding an FIC of <0.3125 (Wu et al. 2011).

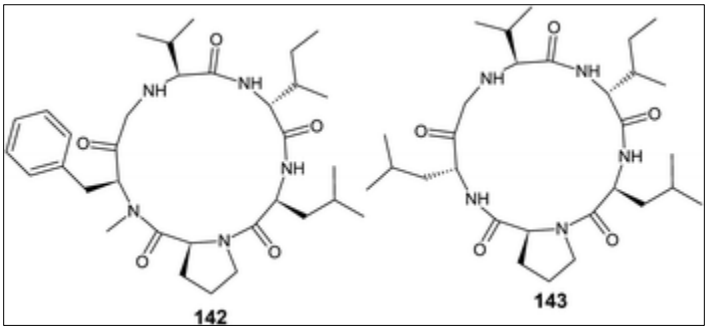

Fig. 9 Structures of new peptide compounds isolated from endolichenic fungi

Allylic compounds

The endolichenic fungus *Massarina* sp. yielded a single novel fatty acid, (11S,12S,13R) 11,13-dihydroxy-12-methyltetradecanoic acid (**144**) (Fig. 10) (Yuan et al. 2015). There have been no reported bioactivity studies or biosynthetic investigations for this metabolite.

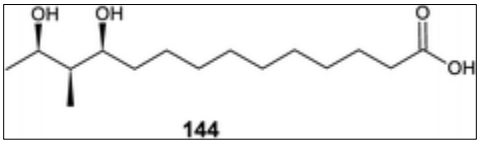

Fig. 10 Structures of the new allylic compound (11S,12S,13R) 11,13-dihydroxy-12-methyltetradecanoic acid, isolated from endolichenic fungi

Conclusions

The 144 new endolichenic fungal metabolites highlighted in this review represent only a small subfraction of endolichenic fungal chemistry. Multiple studies of endolichenic fungi have evidenced bioactivity from various fungal extracts, but have provided incomplete structural identification of the bioactive constituents. Cheon et al. (2013) examined 571 endolichenic fungi for their antifungal properties, identifying four—*Stereocaulon* sp. (1429), *Stereocaulon* sp. (1430), *Cryptosporiopsis* sp. (0156), and *Graphis* sp. (1245)—that possessed high levels of antifungal activity. While several metabolites were identified in the active fractions, none were isolated and confirmed for bioactivity. Similarly, other studies have uncovered endolichenic fungi with potent bioactivity, but complete identification of the metabolites has not yet been reported (Hwang et al. 2011; Kannangara et al. 2009; Kim et al. 2012; Padhi and Tayung 2015).

The new endolichenic metabolites reviewed here, as well as previously described metabolites from these organisms, share many similar carbon skeletons with metabolites produced by endophytic fungi (Kharwar et al. 2011; Kusari et al. 2012; Stierle and Stierle 2015). The overlap between endolichenic and endophytic metabolites is consistent with their biological similarities; there exists considerable overlap in the taxa represented in endolichenic and endophytic fungal strains, and they are believed to perform similar ecological roles for the host organism (Chagnon et al. 2016; U'Ren et al. 2010). However, endolichenic fungal metabolites remain relatively distinct from the natural products produced by lichens individually (Boustie et al. 2011; Romagni and Dayan 2002; Shukla et al. 2010). And despite any similarities, this review has highlighted several instances of metabolites with novel skeletons, including the phaeosphaerins A–F (Li et al. 2012), nodulisporiviridins A–H (Zhao et al. 2015a), Pericoterpenoid A (Wu et al. 2015), Conioxepinols A–D (Wang et al. 2010b), and 6-hydroxy-8-methoxy-3a-methyl-3a,9b-dihydro-3H-furo[3,2-c]isochromene-2,5-dione (Wang et al. 2012), and Coniothiepinols A and B (Wang et al. 2010a). Thus, while endolichenic fungal metabolites do possess some overlap with endophytic fungal natural products, they also possess novel biosynthetic pathways capable of producing novel products.

Endolichenic fungi and other microbial source of natural products maintain a degree of biosynthetic plasticity in producing natural product metabolites. By applying systematic variations in the cultivation parameters (media composition and phase, aeration, pH, temperature, culture vessel, addition of enzyme inhibitors, epigenetic modifiers etc.), it is possible to increase the number of metabolites produced by a fungal (or microbial) source. Altering the culturing conditions increases the metabolomic diversity available to these organisms, and has been termed the "one strain, many compounds" (OSMAC) approach (Bode et al. 2002). Several of the endolichenic fungi have been shown to be responsive to OSMAC approaches, biosynthesizing new metabolites as a result of the variations in culturing. The endolichenic fungal strain *Nodulisporium* sp. (No. 65-12-7-1) was found to produce additional 4-methyl progesteroid analogs when grown in potato-dextrose-broth (PDB) (Zhao et al. 2015c). Three new terpenoids, the tricycloalternarenes F–H were isolated from a Czapek's culture of *Ulocladium* sp. (CGMCC 5507) (Wang et al. 2013a), while PDB cultures of the same endolichenic fungal strain yielded five novel ophiobolane sesterterpenes, the ophiobolins P–T. Neither of these groups of metabolites were detected in the original rice cultures (Wang et al. 2013b). Thus, OSMAC approaches to probing chemical diversity in endolichenic fungi also

have the potential to maximize and further develop the natural products produced by these microorganisms.

Furthermore, the 31 endolichenic fungi whose metabolites have been reported here were collected from a limited number of geographic locations (Fig. 11). It is important to note that this data is only presented for those references (33 of 39) that included either GPS coordinates or a geographical descriptor, and the inclusion of geographic data remains a key metric across all natural product discovery efforts (Leal et al. 2016; Oberlies et al. 2009). With estimates for the global number of currently recognized lichens near 20,000 species (Feuerer and Hawksworth 2007), there remains a vast reservoir for prospective endolichenic fungi that have the potential to provide bioactive natural products.

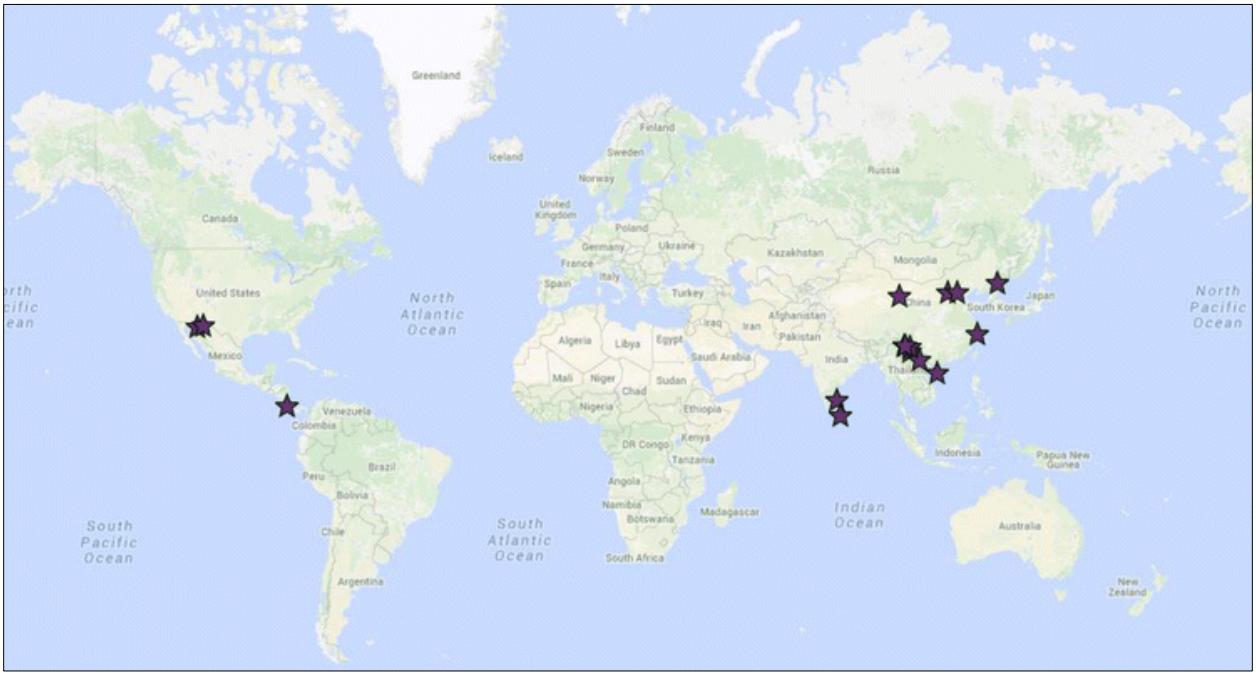

Fig. 11 Geographic locations of metabolite-producing endolichenic fungal strains identified in this review (*Note* this data is only presented for the references that included geographic descriptors) (Ding et al. 2009; Dou et al. 2014; He et al. 2012; Jiao et al. 2015; Kim et al. 2014; Li et al. 2012, 2015a, b, c; Paranagama et al. 2007; Samanthi et al. 2015; Wang et al. 2013a, b, 2012, 2010a, b; Wijeratne et al. 2010, 2012; Wu et al. 2011, 2015; Yang et al. 2012, 2015, 2013; Ye et al. 2013, Zhao et al. 2014, 2015a, b, c; Zheng et al. 2013, 2014)

Further investigations at the genetic, molecular, and population level in this field are needed for a more thorough understanding of host–endolichenic fungi interactions, as well as improved understanding of the ecological role that endolichenic fungi, and their secondary metabolites, play in the symbiosis with and protection of the lichen's photobiont. A recent study on the evolution of fungal metabolic pathways suggests that gene duplication and horizontal gene transfer have acted together in imparting diversity to metabolic gene clusters within the Pezizomycotina. These data acquired from 208 diverse fungal genomes provides further impetus for studying endolichenic fungi for secondary metabolites, since most fungi isolated as

endolichenic belong to the Pezizomycotina (Wisecaver et al. 2014). More research will also aid in creating a comprehensive understanding of the evolutionary origins of endolichenic fungi, as well as the mechanisms and roles of their apparent genetic plasticity in producing secondary metabolites. Understanding the ecological and genetic roles of endolichenic fungi, will aid in identifying highly active metabolite-producing strains.

As a recently discovered reservoir of fungi 'hidden' within host lichens, endolichenic fungi are a potentially rich source of bioactive and chemically novel compounds. While these discoveries are inspirational in uncovering new areas of bioactive natural products, challenges still exist for the future development of endolichenic fungi discovery: (1) frequent rediscovery of known natural products (El-Elimat et al. 2013) (2) technical challenges associated with their purification and structural identification; (3) traditional screening strategies at for bioactive compounds (Kellogg et al. 2016); (4) uncultivable strains as yet another unexploited source of natural products (Nichols et al. 2010). A great deal of effort remains to unearth the potential of endolichenic fungi as natural product producers. However, if the current limitations of methodologies and technologies could be overcame, a new horizon could open up for natural products from endolichenic fungi as novel compounds for the benefit of human health.

Notes

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