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Complete List of Authors:	Zhang, Li-Wen; Biotechnology Research Institute, Chinese Academy of Agricultural Sciences, Fasoyin, Opemipo Esther; Chinese Academy of Agricultural Sciences Biotechnology Research Institute, Xu, Yuquan; Chinese Academy of Agricultural Sciences Biotechnology Research Institute, Molnar, Istvan; University of Arizona, Natural Products Center

Secondary metabolites from Hypocrealean entomopathogenic fungi: Novel bioactive compounds

Liwen Zhang[1], Opemipo Esther Fasoyin[1], István Molnár[2,*], Yuquan Xu[1,*]

[1] Biotechnology Research Institute, The Chinese Academy of Agricultural Sciences, 12 Zhongguancun South Street, Beijing 100081, P.R. China

[2] Southwest Center for Natural Products Research, University of Arizona, 250 E. Valencia Rd., Tucson, AZ 85706, USA.

Abstract

Entomopathogens constitute a unique, specialized trophic subgroup of fungi, most of whose members belong to the order Hypocreales (class Sordariomycetes, phylum Ascomycota). These Hypocrealean Entomopathogenic Fungi (HEF) produce a large variety of secondary metabolites (SMs) and their genomes rank highly for the number of predicted, unique SM biosynthetic gene clusters. SMs from HEF have diverse roles in insect pathogenicity as virulence factors by modulating various interactions between the producer fungus and its insect host. In addition, these SMs also defend the carcass of the prey against opportunistic microbial invaders, mediate intra- and interspecies communication, and mitigate abiotic and biotic stresses. Thus, these SMs contribute to the role of HEF as commercial biopesticides in the context of integrated pest management systems, and provide lead compounds for the development of chemical pesticides for crop protection. These bioactive SMs also underpin the widespread use of certain HEF as nutraceuticals and traditional remedies, and allowed the modern pharmaceutical industry to repurpose some of these molecules as life-saving human medications. Herein, we survey the structures and biological activities of SMs described from HEF from 2014 up to the third quarter of 2019, and summarize new information on the roles of these metabolites in fungal

virulence.

1. Introduction

The order Hypocreales (Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae) features the largest number of entomopathogenic fungal species (476 out of 1,592 species, with only the teleomorphs counted in this extremely diverse order).[1] Many of these Hypocrealean Entomopathogenic Fungi (HEF) have a long history of being investigated as biocontrol agents,[2, 3] with about 170 strains belonging to 12 different species having been developed commercially and marketed so far.[4, 5] HEF are also known to produce a large number of structurally diverse secondary metabolites (SMs) with a remarkable range of bioactivities and potential applications in human and veterinary medicine and agricultural production.[2, 3, 6-9] For example, the immunosuppressant cyclosporine (a SM produced by the HEF *Tolypocladium niveum*) has been widely used for decades to avoid organ rejection following transplant surgery.[10] Another immunosuppressant, Fingolimod (Gilenya; FTY720) that was approved by the FDA (September 2010) and the European Medicines Agency (March 2011) as a new treatment for multiple sclerosis, is derived from myriocin (ISP-I), a SM of the HEF *Isaria sinclairii*.[11]

1.1. Hypocrealean entomopathogenic fungi

The infection cycle of HEF starts with the attachment of infective spores, typically conidia, onto the outer surface of the insect integument.[3] After germination, the hyphae directly penetrate the cuticle of the host using specialized apparatus such as appresoria, and rapidly proliferate as yeast-like cells or hyphal bodies in the hemolymph, eventually killing the host.[12] After utilizing the cadaver as a food source, the fungus produces more infective conidia or resting structures (thick-walled, sexual or asexual resting spores) on the outside surface of the insect integument, which then start the next infection cycle.[13]

Several studies suggested that entomopathogenicity in fungi has evolved multiple

times in the phylum Ascomycota, starting from phytopathogenicity and saprotrophy as the ancient trophic modes.[14, 15] Typical HEF strains belong to the families of Cordycipitaceae, Clavicipitaceae and Ophiocordycipitaceae. The Cordycipitaceae incorporate geographically widespread species from the genera *Beauveria*, *Cordyceps*, and *Isaria*. Species from *Beauveria* along with *Metarhizium* (from the family Clavicipitaceae) provide the most important commercial strains of bio-insecticides; they are known to infect more than 200 species of different insects that are important agricultural pests or act as vectors for human and animal diseases.[4, 16, 17] *Isaria fumosorosea* is also commercially available for the biocontrol of pests such as whitefly.[17] For further information on the major entomopathogenic fungi, their host range and commercial formulations as biopesticides, the reader is referred to the recent review of Singh *et al*. (2016).[4, 16]

Fungal SMs affect insect physiology and behavior in multiple ways. Volatile SMs may act as repellents or attractants, while non-volatile compounds serve as toxins to help to combat the host immune system and eventually kill the insect, or function as deterrents or stimulants[18] to compete with opportunistic pathogens for the nutrient resources of the host.[19] These properties may allow us to repurpose the SMs of HEF into the active ingredients of pesticides, although the production cost of these active ingredients often limits the targeted market and the opportunity for commercial success.[20] To discover novel, commercially useful insecticidal SMs, most research groups utilize bioactivity assays with insect lethality as the readout. Thus, lethal doses expressed as LD_{50} values are frequently reported, unfortunately in most cases without any further mechanistic explanation for the insect mortality observed. Recently, more detailed investigations on the specific physiological processes targeted by fungal SMs have also been conducted.[21, 22]

Importantly, many species of the genus *Cordyceps* and some members of the Ophiocordycipitaceae are also widely used as nutraceuticals and traditional medicines, especially in Asian countries.[9] Biocontrol and TCM (Traditional Chinese Medicine) strains of HEF are rich sources of SMs with a broad range of bioactivities

modulating the hepatic, renal, cardiovascular, immune and nervous systems, while many display prominent anticancer activity.[23] Thus, investigations into the "secondary metabolome" or "parvome"[24] of HEF not only elucidate the mechanisms of fungus-host interactions, but also make it possible to better target and further develop fungal SMs for agrochemical and veterinary or human medical applications.

1.2. Scope of the review

This review covers the structurally characterized SMs of HEF, primarily polyketides (PKs), nonribosomal peptides (NRPs), alkaloids, terpenoids and their hybrids, concentrating on the period of **2013** to the third quarter of **2019**. It is an update to complement two previous reviews,[2, 3] and summarizes the structures of the newly discovered compounds, their identified biological activities, potential utilities in medicine and roles in entomopathogenesis (if known). The use of genomics to discover novel SMs from HEF and to clarify their biosynthesis is described in a companion review in this issue.

As with our previous review,[3] we concentrate on SMs from fungi from the Clavicipitaceae, Cordycipitaceae and Ophiocordycipitaceae families where the entomopathogenic trophic mode of the fungus is apparent, or at least highly likely from the descriptions in the primary articles. Thus, we discuss newly discovered SMs from HEF species belonging to the genera *Aschersonia* (*Hypocrella*), *Beauveria, Conoideocrella* (*Torrubiella*), *Cordyceps*, *Gibellula, Hevansia* (formerly *Akanthomyces*), *Hirsutella, Isaria, Lecanicillium, Metarhizium, Moelleriella, Ophiocordyceps, Paecilomyces, Tolypocladium, Trichothecium,* and *Verticillium*. SMs from strains of fungi with primarily phytopathogenic, sapropbiotic, mycoparasitic or necrophytic trophic modes, or those that are likely opportunistic insect pathogens (such as those fungi that gain entry to the insect hemolymph without breaching the cuticle) are not included in this review.[25]

The genus *Cordyceps* is an especially prolific and widely studied resource for SM discovery, with over 200 compounds described that belong to the nucleosides, sterols,

flavonoids, cyclic peptides, phenolics, polyketides and alkaloids. Some of these compounds show diverse bioactivities, including anti-inflammatory, antitumor, antimalarial, and antifungal activities. A recent review[9] thoroughly summarized the SMs produced by *Cordyceps* spp., thus in this review we cover only those *Cordyceps* SMs that show the most interesting bioactivities or those that have not been mentioned by Olatunji *et al*. (2018).

Taxonomic names of fungi featured in this review follow those in Mycobank (www.mycobank.org).

2. Emerging methodologies expand SM discovery

Recent genome/transcriptome sequencing of many fungal species, including HEF, have confirmed that most SM biosynthetic gene clusters (BGCs) are silent or expressed only at very low levels under standard laboratory conditions. Thus, the capacity of fungi to produce SMs far exceeds what is manifest in standard fermentations.[26-28] Even SMs from the expressed BGCs routinely escape detection when dereplication is guided by one or just a few conventional bioactivity screens. In addition, many bioactivity assays are only sensitive enough to detect the most abundant bioactive metabolites in the crude extract. Therefore, strategies to activate the silent or weakly expressed BGCs are critical to obtain new products from existing fungal isolates. These new strategies include elicitation of SM production by interspecies communication; epigenetic modulation of gene expression; manipulation of pleiotropic regulatory circuits or cluster-specific regulators; direct modulation of BGC expression by promoter engineering; and heterologous expression of BGCs.[28-32] Some of these novel methods have been successfully applied to HEF recently and resulted in the isolation of new products.

2.1. Epigenetic modulation of BGC expression

Epigenetic modifications such as histone acetylation and deacetylation play an important role in the regulation of gene expression in different kingdoms of

eukaryotic organisms ranging from fungi to plants and animals.[29] Epigenetic regulation of gene expression is widely investigated as a target for curing human diseases, such as the use of KAT6HATs in regulating cell proliferation.[33] In fungi, histone alkylation (such as methylation or acetylation) status was found to correlate with the global regulation of SM production.[34-36] The manipulation of epigenetic regulators can induce or repress the production of multiple SMs through chromatin remodeling that affects the BGCs located in heterochromatic regions.[26] For example, overexpression of the histone-4 acetyltransferase EsaA increased SM production in *A. nidulans*,[37] while the loss of the histone acetyltransferase AflGcnE in *A. flavus* led to increased repression of the aflatoxin BGC.[38] In contrast, deletion of histone H3 lysine-4 methyltransferases led to the activation of "silent" SM gene clusters or the upregulation of weakly expressed BGCs.[29] As for HEF, both molecular genetics-based and chemical approaches targeting histone and DNA posttranslational modification processes have succeeded in the activation of SM BGCs. For example, deletion of a histone acetyltransferase gene in *M. robertsii* induced the production of 11 new SMs, including eight isocoumarin derivatives (meromusides A−H) and two nonribosomal peptides (meromutides A and B).[29] Addition of epigenetic modifying agents, such as inhibitors of histone deacetylase and DNA methyltransferase have also induced the transcription of silent BGCs to afford a variety of SMs.[39-43] The concurrent addition of both histone deacetylase and DNA methyltransferase inhibitors can further change the product profiles and activate the production of new SMs.[44, 45] These approaches may be applied in a semi-high throughput context, but are empirical in nature and offer little predictability.[27]

2.2. Manipulation of transcriptional regulatory networks

The production of SMs is controlled by a complex, multilevel regulatory network that harmonizes the expression of BGCs with other metabolic, morphogenetic and developmental processes as a coordinated response to the external and internal environment of the fungal cells.[26] This regulatory network involves "global",

pleiotropic transcriptional regulators orchestrating multiple metabolic or developmental subroutines; mid-level transcriptional regulators that are modulating a smaller subset of processes; and dedicated, cluster-specific transcriptional regulators directing a selected BGC. Our increasing understanding of this regulatory network allows the development of successful strategies to activate silent BGCs.[26] The best known examples include the manipulation of the VelB/VeA/LaeA complex in *Aspergillus* spp,[46, 47] which led to the discovery of compounds such as 3-methoxyporriolide.[48] Such global and pathway-specific regulators were also found to correlate with HEF SM production. The loss of BbPacC, an important transcription factor in *B. bassiana* for ambient pH response, resulted in the identification of the yellow-colored pigment bassianolone B, while eliminated the production of the insecticidal compound dipicolinic acid.[49] Bassianolone B was previously identified as an intermediate of the cephalosporolide pathway.[50, 51] Under sulfur limiting conditions, cephalosporolide E and F were also produced by the fungus.[50] Pathway-specific transcriptional factors are often observed to directly regulate the expression of the BGCs and the consequent production of compounds in HEF, such as the basic leucine zipper (bZIP)-type regulator SimL in cyclosporine BGCs,[22] OpS3 in oosporein BGCs,[21] and Bea4 and Yy1 in beauvericin BGCs,[52] emphasizing the potential for discovering new SMs through manipulation of key regulatory elements.

2.3. Heterologous expression

As an alternative to manipulating global or cluster-specific regulators, heterologous expression of complete BGCs in "domesticated" host organisms allows the bypassing of native regulatory circuits and the production of novel SMs in a more controlled fashion.[10] The choice of hosts extends from unicellular model organisms such as *Saccharomyces* or other yeasts to well-characterized filamentous fungi such as *Aspergillus* spp. Thus, Ishiuchi *et al*. (2012) developed a yeast-based platform for the heterologous expression of PKS and NRPS genes derived from higher fungi. To aid the cloning of large synthase or synthetase genes (typically 5 to 20 kb), their

method encompasses an expression vector that can be assembled with the PKS or NRPS gene of interest using overlap extension PCR and yeast-based homologous recombination in an engineered *S. cerevisiae* strain.[53] *Aspergillus nidulans* is a genetic model species among filamentous fungi that is better suited for the biosynthesis of complex SMs than *S. cerevisiae*. Nielsen et al. (2013)[54] developed a smart system for selectable marker recycling in this host based on homologous integration of the incoming gene cassette into the *IS1* locus that supports high levels of expression. This approach allowed the stepwise transfer of all 13 genes of the *A. terreus* geodin BGC into *A. nidulans* and the dissection of the biosynthetic steps involved.[54] Heterologous expression of three genes from the HEF *M. robertsii* ARSEF 23 in *A. nidulans* A1145, including a polyketide synthase, a prenyltransferase and a geranylgeranyl diphosphate synthase led to the production of an α-pyrone-fused non-cyclic diterpene, the expected intermediate of the meroterpenes subglutinols C and D.[55] Heterologous expression also confirmed the function of a series of HEF BGCs, including those that are responsible for the production of tenellin from *B. bassiana*,[56] and trichothecene in *B. bassiana* and *Cordyceps confragosa*.[57] Heterologous expression strategies can also simplify the identification of novel metabolites, especially when the genetic or microbiological manipulation of the host is problematic. However, this method is still best suited to relatively small gene clusters (<40 kb) and may miss some of the tailoring reactions that provide the fully elaborated SM in the native producer. This may be the situation when those tailoring reactions are catalyzed by enzymes whose encoding genes are not physically clustered with the rest of the BGC, or if those reactions require substrates that are not available, or cofactors that are limiting in the surrogate host.

2.4. Less investigated species

In addition to novel methods aimed at the silent BGCs, efforts to characterize the parvome of less-investigated HEF also remain important. Thus, in addition to the well characterized *Beauveria*, *Metarhizium*, and *Cordyceps* spp., less well-known HEF,

including new species, have recently been included in the search for bioactive SMs. For example, the parasites of spiders such as *Hevansia* (formerly *Akanthomyces) novoguineensis* (Cordycipitaceae) have received little attention as sources of natural products until recently.[58-60] Similarly, HEF that specifically attack scale insects such as *Conoideocrella luteorostrata* and *Conoideocrella tenuis* (formerly *Torrubiella luteorostrata* and *To. tenuis*, respectively) from the Clavicipitaceae have also been shown to produce a wealth of SMs.[61] In addition to new insect hosts, more exotic habitats such as the marine sediment[62-65] or the gut microbiome[66] have also been explored for new HEF species and their associated SMs.

3. Newly discovered secondary metabolites from HEF

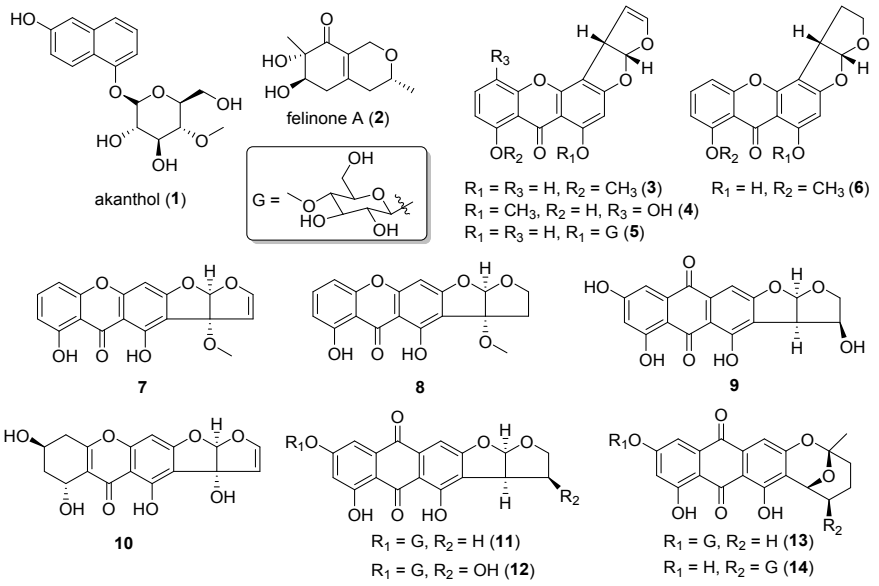
3.1. Polyketides

Fungal polyketide synthases (PKSs) assemble polyketide (PK) scaffolds by the sequential condensation of carboxylic acid monomers using a single set of enzymatic domains that act in an iterative manner, and follow a still little understood biosynthetic program.[67-70] Typically, these PK scaffolds are further modified by a series of tailoring enzymes to afford the final, mature PK products with diverse structures and functions. Fungal iterative PKSs (and by extension, their PK products) may be classified into three types according to the domain composition of these multienzymes. The so-called "core domains", that is the ketosynthase (KS), the malonyl/acyl-CoA:ACP transacylase (AT), and the acyl carrier protein (ACP) domains are universally present in all three types of fungal PKSs and are responsible for the growth of the PK chain by successive decarboxylative Claisen condensations. In addition to the core domains, highly reducing PKSs (hrPKS) also include a *β*-ketoacyl reductase (KR), a dehydratase (DH), and an enoyl reductase (ER) domain that yield alcohol, olefin or alkane moieties, respectively, in a progressive reduction sequence whose extent (and the configuration of the resulting stereocenter, if any) is programmed for every PK chain extension cycle. Partially reducing PKSs (prPKSs) feature KS domains that clade with the methylsalicylic acid synthase PKSs

(MSAS) in a group distinct to hrPKS. These synthases lack the ER domain and thus are unable to produce fully saturated carbon-carbon bonds. Finally, the non-reducing PKSs (nrPKSs) lack reducing domains, but regularly incorporate a starter acyltransferase (SAT) domain for selecting the initial monomer, and a product template (PT) domain that catalyzes a regiospecific aldol condensation to form the first aromatic ring. Many PKSs also feature a chain termination domain; for those that lack this, additional enzymes routinely encoded in the BGC act to release the PK product. In addition, *C*-methyltransferase (CMT) domains that are programmed to methylate some of the α-carbons are also commonly found in fungal PKSs. The mechanistic enzymology of these domains has been reviewed in detail.[67, 69]

3.1.1. Nonreduced polyketides

A glycosylated β-naphthol, akanthol (**1**) was found to be synthesized by the spider-associated fungus, *Hevansia* (formerly *Akanthomyces) novoguineensis* BCC47894 (Cordycipitaceae, Ascomycota). Akanthol was not active in antimicrobial, cytotoxic, anti-biofilm, and nematicidal assays even at the highest tested concentrations of 300, 37, 33.3, and 100 μg/mL, respectively.[59] Although antimicrobial agents with the 2-naphthol moiety are well known, glycosylation of the 2-naphthol scaffold apparently leads to the loss of such activities.[59] While glycosylation is widespread amongst SMs, it is remarkable that those bearing the 4-*O*-methylglucose moiety (as seen in akanthol) were mostly isolated from HEF.[3, 58, 59, 71-74] The glucosyltransferase – methyltransferase gene pair that encodes the enzymes that are responsible for "decorating" various phenolic compounds with the 4-*O*-methylglucose moiety has recently been discovered in *Beauveria bassiana*. This gene pair is unique to HEF species, leading to the characteristic glycosylated SM profiles of these organisms.[75]

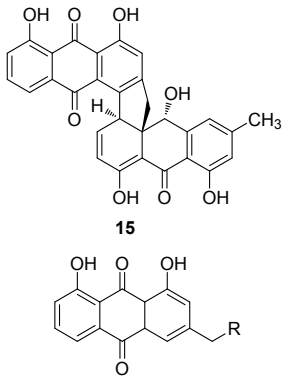


A new *O*-containing pentaketide, felinone A (**2**) was isolated from the culture of *Beauveria felina* EN-135.[64] Although this particular strain was isolated from a marine bryozoan, other *B. felina* strains are frequently obtained as insect pathogens in terrestrial environments. Felinone **2** was weakly toxic to the brine shrimp with a lethal rate of 61.4% at a concentration of 100 μg/mL.[64]

Xanthone and anthraquinone-type compounds are an important class of mycotoxins.[73] Seven new xanthones **3**-**9**, one hydroxanthone **10**, and four new anthraquinones **11**-**14** were isolated from the scale insect fungus *Aschersonia coffeae* BCC 28712[73] and *A. marginata* BCC 28721,[76] together with known sterigmatocystin, sterigmatin, averufin, aflatoxin, and paeciloquinone analogues. The crude extract of both strains exhibited cytotoxicity against MCF-7 (human breast cancer), KB (human oral epidermoid carcinoma), and NCI-H187 (human small-cell lung cancer) cells, while the crude extract of BCC 28712 also showed antimalarial activity. When pure compounds were tested, **3**-**5** and **12**-**13** displayed cytotoxic activity against NCI-H187 with IC_{50} of 1.17, 12.93, 2.86, 8.06 and 5.12 μg/mL (c.f ellipticine as the positive control, IC_{50} of 1.31 μg/mL). Compounds **3**-**5** were also toxic to Vero cells (African green monkey kidney fibroblast) with IC_{50} of 0.34, 1.40, 0.33 μg/mL, respectively (c.f ellipticine as the positive control, IC_{50} of 1.29 μg/mL).[73] In addition, xanthone **4** and anthraquinones **12** and **13** also displayed weak antimalarial activity with IC_{50} of 8.54,

3.88 and 1.60 μg/mL, respectively (c.f. dihydroartemisinin as the positive control with IC_{50} of 0.0005 μg/mL).[73]

Two new anthraquinone dimers, torrubiellins A (**15**) and B (**16**), were isolated from a culture of *Torrubiella* sp. BCC 28517, alongside three known anthraquinone compounds, chrysophanol (**17**), aloe-emodin (**18**) and emodin. *Torrubiella* sp. BCC 28517 is a leafhopper-pathogenic fungus of the family Cordycipitaceae. Compound **16** possessed various antimicrobial activities: antimalarial (*Plasmodium falciparum*, IC_{50} of 0.33 μg/mL, c.f mefloquine as the positive control with an IC_{50} of 0.013 μg/mL); antifungal (*Candida albicans*, IC_{50} of 1.66 μg/mL, c.f. amphotericin B as the positive control with an IC_{50} of 0.072 μg/mL) and antibacterial activities (*Bacillus cereus*, IC_{50} of 6.25 μg/mL, c.f. vancomycin hydrochloride as the positive control with an IC_{50} of 4.0 μg/mL). Compound **16** also showed cytotoxicity against cancer cell lines KB, NCI-H187, and MCF-7 (IC_{50} 0.48, 0.20 and 3.20 μg/mL, respectively; c.f. doxorubicin hydrochloride as the positive control with IC_{50} of 0.30, 0.045 and 6.47 μg/mL).[77]



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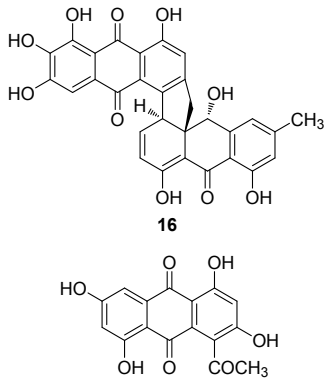


16

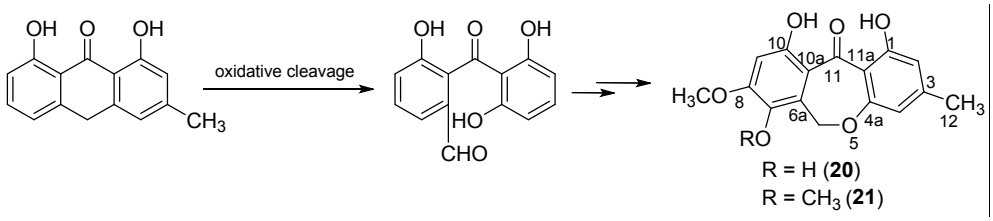
R=H (**17**)
R=OH (**18**)

19

One anthraquinone product (**19**) was identified from *M. robertsii* while searching for a BGC for melanin production. Melanin increases resistance to abiotic stresses and is an important virulence determinant in different pathogenic fungi.[4, 78] In spite of the characteristic dark green color of *M. robertsii* colonies, similarity searches failed to locate a typical melanin BGC in the published genome sequence of this fungus.[78, 79] Nevertheless, two separate nrPKSs, MrPKS1 (MAA_07745) and MrPKS2

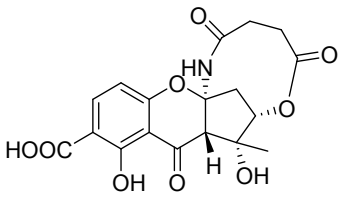
(MAA_03239) were further investigated in *M. robertsii* because they both showed sequence similarities to PKSs involved in pigment production in other fungi.[79] While MrPKS1 and MrPKS2 are likely paralogs, they have evolved to yield different SMs that perform different biological functions, and their encoding genes (*mrpks1* and *mrpks2*, respectively) show different patterns of expression.[78] *Mrpks1* is upregulated during conidiation, and the SM product of the *mrpks1* BGC is a green conidial pigment that helps to protect the fungus against UV radiation and heat and cold stresses.[78] Heterologous expression of *mrpks1* in *A. nidulans* yielded the anthraquinone analogue **19**.[78] The paralogous nrPKS, *mrpks2* was upregulated during cuticle penetration and appressoria formation,[79] with gene deletions implicating MrPks2 in the virulence of *M. robertsii* against insects. However, no new product was detected when *mrpks2* was heterologously expressed in *A. nidulans*.[78]

A new dibenzo[*b,e*]oxepinone, chaetone G (**20**), alongside a known analogue (**21**) were isolated from *Aschersonia luteola* BCC 31749 grown in potato dextrose broth.[80] Dibenzo[*b,e*]oxepinones are rare secondary metabolites of the benzophenone class, and display various biological activities such as antibacterial, antifungal, antitumor, and cytotoxic activities. Dibenzo[*b,e*]oxepinones such as arugosins A–E had previously been isolated from *Aspergillus* spp.; arugosin F was found in *Ascodesmis sphaerospora* cultures; arugosin G was obtained from *Emericella nidulans* var. *acristata*; and chaetones A–F were isolated from *Chaetomium* sp. However, chaetone G is the first member of this class of SM to be identified from a HEF.[80-82] Chaetone G was proposed to be biosynthesized from an anthrone scaffold that undergoes oxidative cleavage, followed by intramolecular condensation (Scheme 1).[80] However, the closely related arugosin J from *Xylaria* sp. was proposed to originate from the condensation of two molecules of orsellinic acid.[83] Compounds **20** and **21** was evaluated in antibacterial and cytotoxicity assays but showed no activity against *B. cereus* or NCI-H187 and Vero cells.[80]



Scheme 1. Proposed biosynthesis of compounds 20 and 21. Adapted from Kornsakulkarn *et al*.,[80] with permission (Copyright 2016, Elsevier).

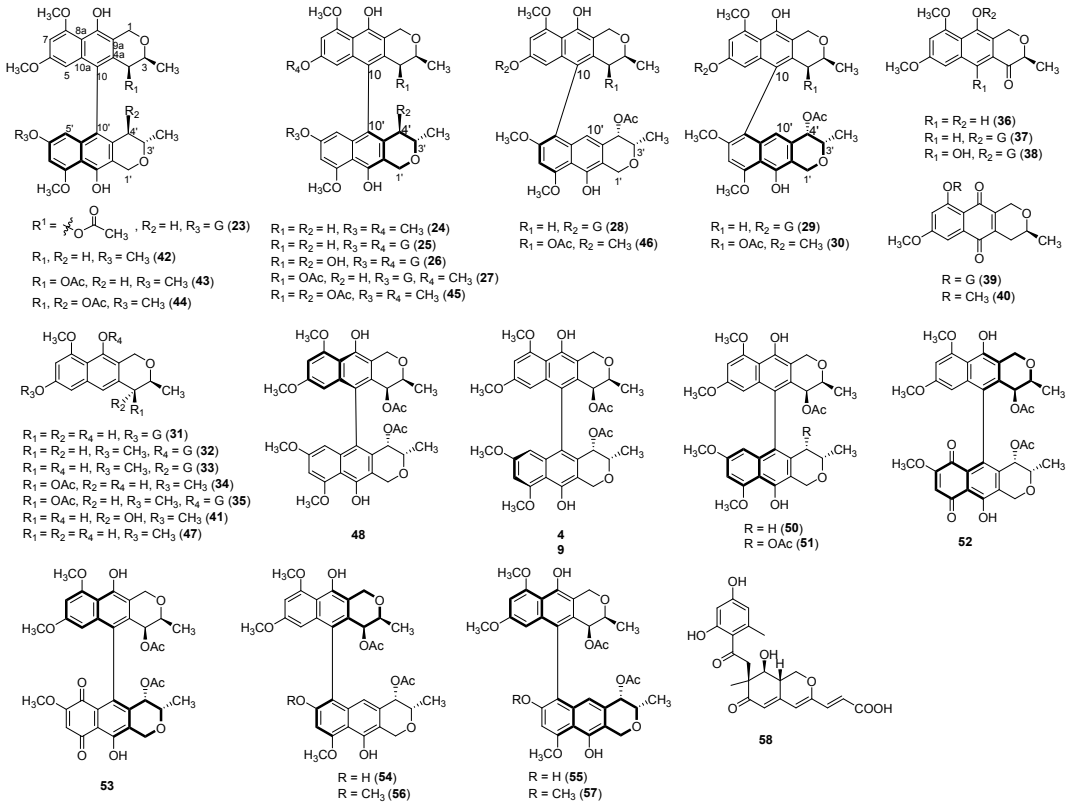
Cryptosporioptide A (**22**), a ring-contracted xanthone with an *N*-succinyl aminal bridge was isolated from *Cordyceps gracilioides*.[84] Related cryptosporioptides with a malonate bridge, and those with only the xanthone-derived core and their dimers, were previously isolated from *Cryptosporiopsis* sp. 8999,[85, 86] and their biosynthesis has recently been elucidated.[87] Compound **22** displays *in vitro* inhibitory activity against the protein tyrosine phosphatases PTP1B, SHP2, CDC25B and SHP1 in the 5 to 8 μM range.[84]



22

Naphthopyran and naphthopyrone SM based on a nonreduced heptaketide framework produced by nrPKS enzymes are common constituents of the HEF parvome.[3] The dimeric naphthopyran bioxanthracenes and their monomers were isolated from static submerged fermentations of *Cordyceps pseudomilitaris* as well as other *Cordyceps* strains and an undescribed *Verticillium* sp.[3] These compounds showed antimalarial and neuroprotective activities. Thirteen additional bioxanthracenes, of which eight (**23-30**) are new, as well as twelve oxanthracenes and their analogues, including nine new (**31-39**) were isolated from the HEF *Conoideocrella luteorostrata* BCC 31648 (Clavicipitaceae).[74] Compounds **36-38** are naphthopyranones, and **39** is a pyranonaphthoquinone. The same fungus also yielded two new quinones **40**[88] and **41**[89], and the known compounds **42-47.**[71, 90, 91] *Co.*

luteorostrata BCC 31648 is the teleomorph of *Paecilomyces cinnamomeus* that is pathogenic to scale insects.



Two new bioxanthracenes (**48** and **49**) were isolated from a culture of *Conoideocrella tenuis* BCC 18627, another scale insect pathogen, together with the known bioxanthracenes ES-242-1 (**50**) and ES-242-2 (**51**).[71] A different strain, *Conoideocrella tenuis* BCC 44534 produced ten bioxanthracene analogues, including two new quinone derivatives of bioxanthracenes, conoideocrellones A (**52**) and B (**53**), two new bioxanthracenes (**54** and **55**), and four known compounds: **56**, **57**, ES242-2 (**51**) and its atropisomer.[61]

Bioxanthracenes such as the ES242 are known to exhibit antimalarial activity.[92] Correspondingly, compound **55** was weakly active against *Plasmodium falciparum* K1 (IC_{50} of 6.6 μg/mL, c.f. mefloquine hydrochloride as the positive control with an IC_{50} of 0.02 μg/mL), and showed no cytotoxicity against KB, MCF-7, NCI-H187 and Vero cells up to 50 μg/mL (c.f. ellipticine as the positive control with IC_{50} of 1.9-4.0

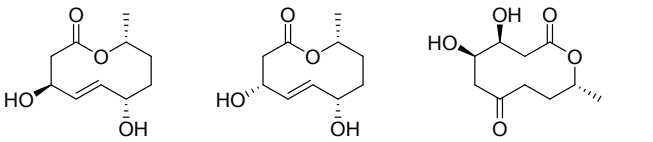
μg/mL). Compounds **52** and **54** showed no antimalarial activity but exhibited weak cytotoxicity. Compounds **36**, **39**, and **40** exhibited both weak antimalarial and cytotoxic activities, while compounds **34**, **37** and **41** were only effective against MCF-7 and NCI-H187 cells. Compounds **24**, **31**, and **33** were only active against NCI-H187 cells. Compound **34** was unique in showing toxicity against KB cells only[74]. Compounds **48** and **49** showed only weak antimalarial activity (*Plasmodium falciparum* K1), but no antimycobacterial, antiviral, and cytotoxic effects.[71]

A new azaphilone dihydroxymethylbenzoate ester, pinophilin C (**58**) was isolated from *Cordyceps gracilioides*.[84] Pinophilins A and B that had been identified from the seaweed-associated fungus *Penicillium pinophilum* are inhibitors of mammalian A-, B-, and Y-family DNA polymerases and arrest human cancer cell proliferation.[93] Pinophilin C inhibited the protein tyrosine phosphatases PTP1B, SHP2, CDC25B, LAR and SHP1 in the 3-8 μM range.[84]

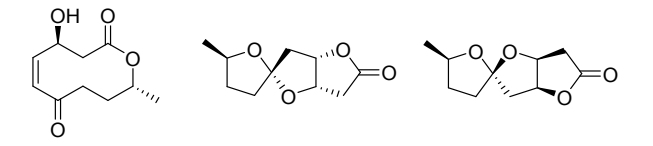
3.1.2. Partially reduced and highly reduced polyketides

Decanolides are abundant ten-membered lactones frequently produced by HEF.[94-96] This family of natural products displays a variety of biological activities, ranging from antimicrobial (antibacterial and antifungal) activities to the inhibition of cholesterol biosynthesis.[95] Besides the macrolactone core, they share a methyl group at C-9, but differ in the number and nature of oxygen functionalities and the degree of unsaturation, and are often subject to extensive post-PKS modifications. Tenuipyrone (**65**) along with two known decanolides, cephalosporolide B (**62**) and cephalosporolide F (**64**) were produced by *Isaria tenuipes* upon cultivation in the presence of the histone deacetylase inhibitor suberoyl bishydroxamate (SBHA), and a DNA methyltransferase inhibitor, RG-108.[45] Tenuipyrone (**65**) features an unprecedented tetracyclic ring system bearing a spiroketal moiety. A mixture of decarestrictine C1 (**59**) and C2 (**60**)[97] and cephalosporolide G (**61**)[51] were obtained from *Cordyceps* sp. NBRC 106954 grown in PSA medium.[23] *Cordyceps* sp. NBRC 106954 was isolated in Japan from a fruiting body occurring on a larva of the cicada

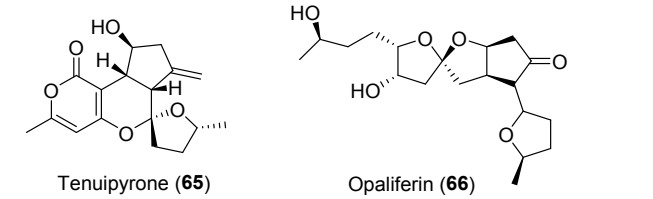
Meimuna opalifera. The same HEF also yielded a PK with a novel C19 skeleton, opaliferin (**66**), featuring a cyclopentanone and tetrahydrofuran moiety connected by an external double bond.[23] Opaliferin is only the second example of this kind of a structure, following oudenone, a tyrosine hydroxylase inhibitor from the fungus *Oudemansiella (Hymenopellis*) *radicata* (Basidiomycota, Agaricales).[98] Opaliferin showed weak toxicity against cells of HSC2 (human oral squamous carcinoma), HeLa (human epithelial adenocarcinoma), and RERF-LC-KJ (human lung adenocarcinoma) but no significant antitrypanosomal or antimalarial activities.



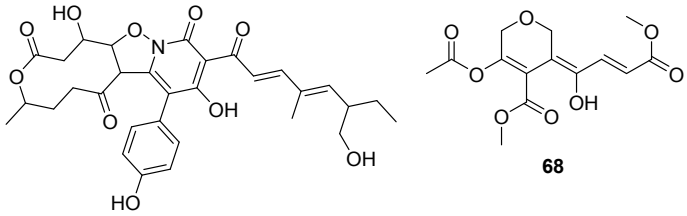
Decarestrictine C_1 (**59**) Decarestrictine C_2 (**60**) Cephalosporolide G (**61**)



Cephalosporolide B (**62**) Cephalosporolide E (**63**) Cephalosporolide F (**64**)



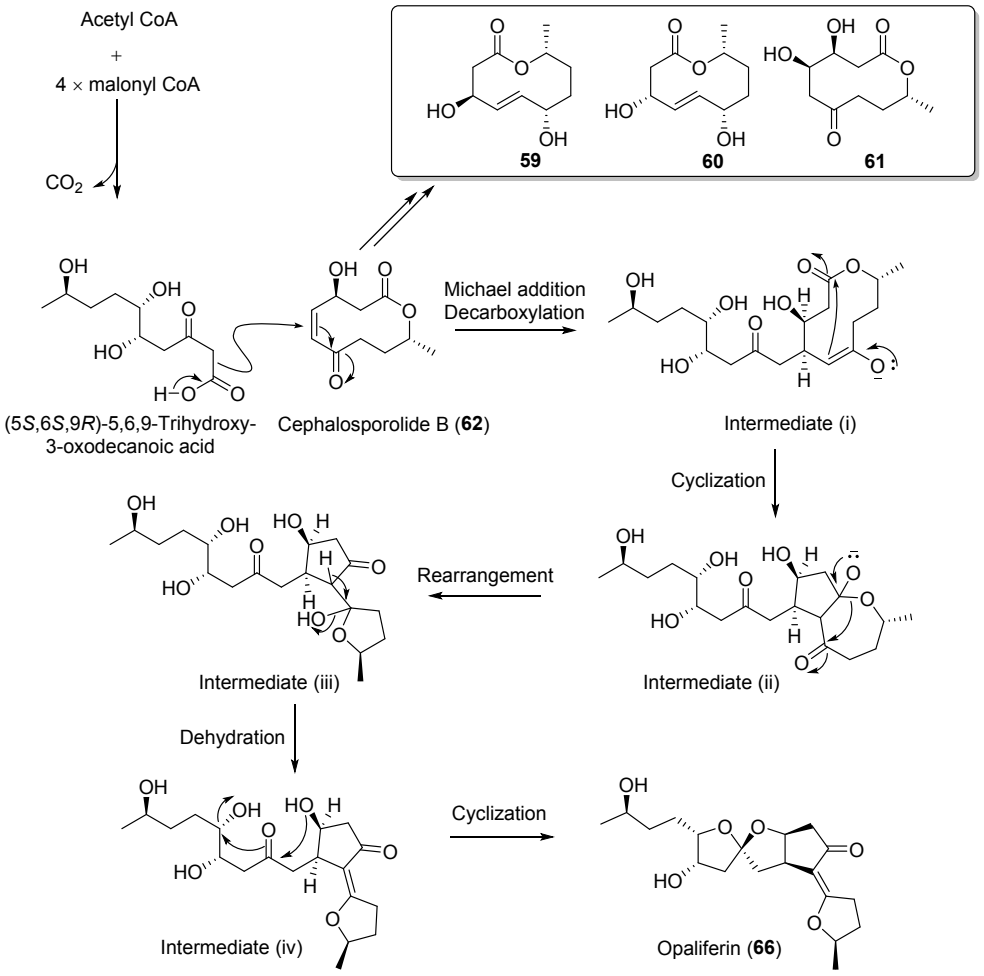
Tenuipyrone (**65**) Opaliferin (**66**)



Pyridomacrolidin (**67**) **68**

The biosynthetic route from cephalosporolide B (**62**) to opaliferin (**65**) was proposed as shown in Scheme 2.[23] Based on the chemical synthesis route, cephalosporolide B **62** can also be the biosynthetic precursor for cephalosporolides G (**61**), C, E (**63**), F (**64**),[51, 99] and other compounds with a related polyketide skeleton such as tenuipyrone (**65**),[45, 99] or pyridomacrolidin (**67**) that features a composite scaffold derived from a decanolide and an acyltetramic acid.[100, 101] During opaliferin (**66**) biosynthesis (Scheme 2),[23] Michael addition between cephalosporolide B **62** and

(5*S*,6*S*,9*R*)-5,6,9-trihydroxy-3-oxodecanoic acid may be followed by decarboxylation to yield the proposed intermediate (i). This compound may undergo intramolecular cyclization through Claisen condensation to afford the 2*H*-cyclopent[*b*]oxepin intermediate (ii). A subsequent rearrangement may provide intermediate (iii) whose dehydration could afford intermediate (iv). Finally, spiro-cyclization may yield opaliferin (**66**).[23]



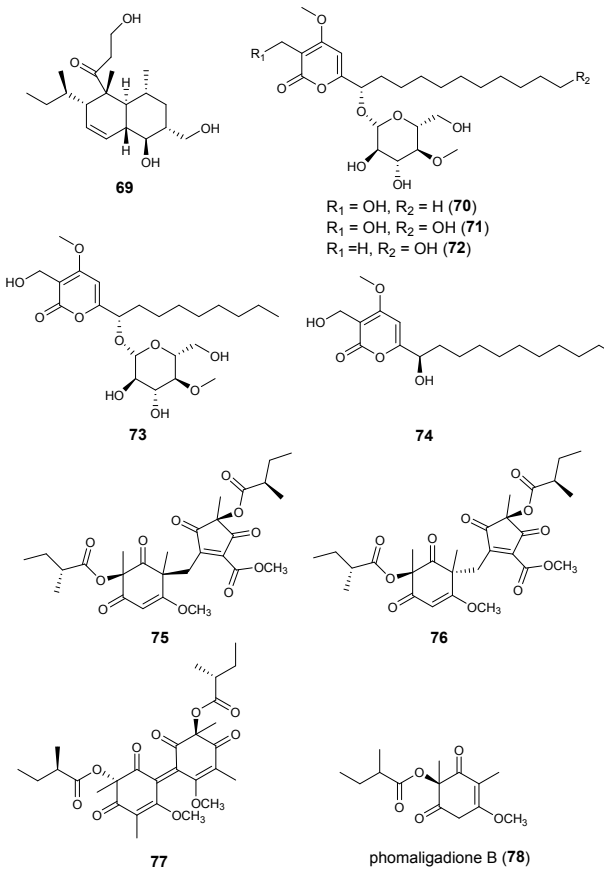
Scheme 2. Proposed pathway for the biosynthesis of decanolides and opaliferin. Adapted from Grudniewska *et al*.[23] with permission (Copyright 2014, American Chemical Society).

Isariketide (**68**) was obtained from a marine isolate (KMM 4639) of the HEF *I.*

felina.[102] Isariketide (**68**) exhibited selective toxicity against THP-1 (human acute monocytic leukemia) and HL-60 (human promyeloblast) cells with an IC_{50} of 37.4 μM and 4.3 μM. These activities were comparable to that of the positive control, cisplatin (IC_{50} of 80.6 and 2.28 μM, respectively). Importantly, compound **68** was neither cytotoxic toward non-transformed CD-I mouse splenocytes nor membranolytic to erythrocytes up to 100 μM.[102]

11-norbetaenone (**69**), a new betaenone compound with a decalin core was isolated from the culture broth of the HEF *Lecanicillium antillanum*.[103] 11-norbetaenone (**69**), a product of a putative hrPKS-containing BGC,[104] displayed significant anti-angiogenic effects against human endothelial progenitor cells by suppressing tube formation. It was inactive in several other bioassays, including anti-inflammatory assays (inhibition of superoxide generation and elastase release); antimicrobial assays (against methicillin-resistant *Staphylococcus aureus*); and cytotoxicity assays against HepG2 (human hepatocellular carcinoma), MDA-MB231 (human epithelial adenocarcinoma), and A549 (human epithelial carcinoma) cells.[103]

Four new glycosylated acyl α-pyrones, akanthopyrones A–D (**70–73**) were isolated from a culture of the spider-infecting fungus *Hevansia* (formerly *Akanthomyces*) *novoguineensis*.[58] The aglycone of akanthopyrone A (**70**) is identical to the previously reported dalsymbiopyrone (**74**) from *Daldinia* sp. (Sordariomycetes, Hypoxylaceae).[105] Akanthopyrone A showed weak antibiotic activity against *B. subtilis* DSM10 and cytotoxicity against the HeLa cell line KB-3-1, while akanthopyrone D (**73**) displayed weak antifungal activity against *Candida tenuis* MUCL 29892. None of these akanthopyrone congeners exhibited anti-biofilm or nematicidal activities.[58]

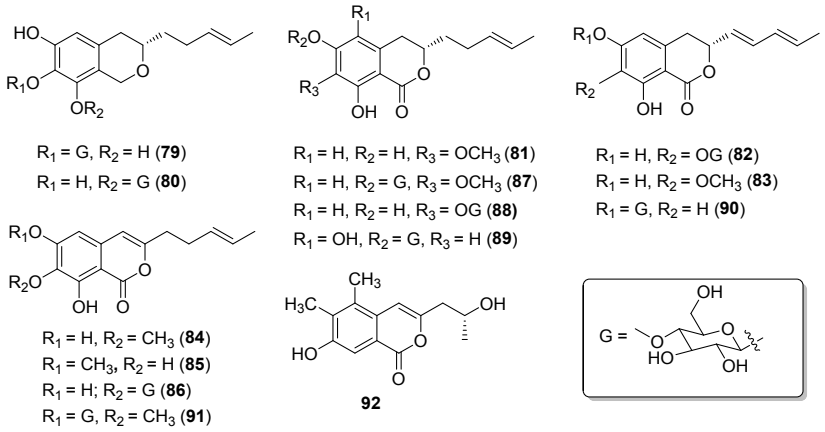


Two novel epimeric polyketides, beauvetetraones A–B (**75-76**), together with the dimeric beauvetetraone C (**77**), all derived from phomaligadione (**78**),[106] were isolated from static liquid cultures of *B. bassiana* JMRC ST000047.[107] Beauvetetraone C was proposed to derive by the oxidative homodimerization of two phomaligadione B (**78**) units. Meanwhile, beauvetetraones A and B with the unprecedented methylene-bridged phloroglucinol skeleton may be formed by Michael addition of two phomaligadione B-derived units, where one of the units has undergone extensive (Favorskii-type) oxidative rearrangements. Compounds **75-77** showed no antifungal activity and no significant cytotoxicity against four human breast cancer cell lines (Bt549, HCC70, MDA-MB-231 and MDA-MB-468, IC_{50} values in the range of 61.9–82.4 μM). Considering the redox-active structures of beauvetetraones, these compounds may be involved in the oxidative stress tolerance of the producer organism, especially during the insect infection process.

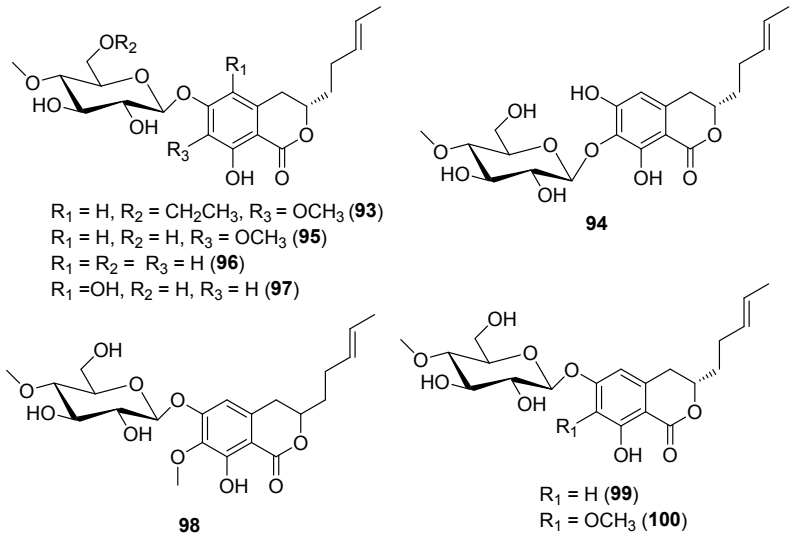
3.1.3. Polyketides from collaborating PKS systems

While the carbon skeleton of most fungal PK compounds is biosynthesized by a single PKS, BGCs featuring two PKSs have also been characterized in fungi. In these collaborative systems, the PK product of one PKS serves as the starter unit for the second PKS (sequential, processive collaborative systems as with those that yield benzenediol lactones and some azaphilones such as asperfuranone).[108, 109] Alternatively, the fully formed PK products of the two PKSs are fused to yield composite products (parallel, convergent collaborative systems such as those that afford lovastatin[110] or chaetoviridin[111]). The akanthopyrones described in the previous paragraph may be produced by a single hrPKS, but they may conceivable derive from a sequential collaborating system that features a hrPKS (or fatty acyl synthase, FAS) producing the alkyl tail, and an nrPKS yielding the pyrone ring.

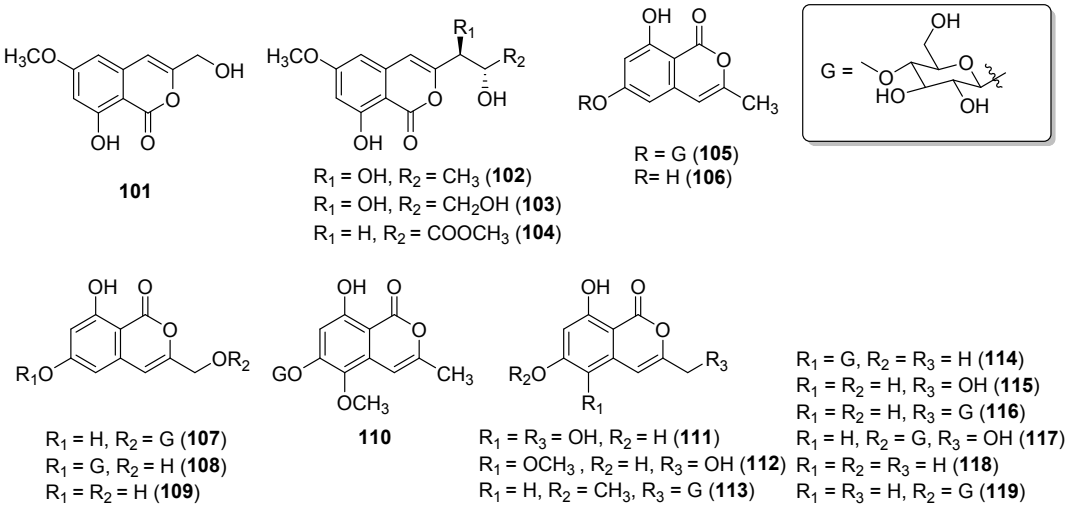
Eight acyl isocoumarins, meromusides A−H (**79**-**86**), were discovered as a result of the deletion of the histone acetyltransferase (HAT) gene in *M. robertsii* (previously known as *M. anisopliae*).[29] *M. robertsii* has been investigated for SM production under various culture conditions and in different developmental stages,[2, 3] yielding nonribosomal peptides (NRPs) such as the destruxins,[112] serinocyclins[113, 114] and metachelins;[115] hybrid PK-NRP products such as NG39x[116] and the cytochalasins;[29] terpenoids such as helvolic acid[117] and ovalicin;[118] and alkaloids such as swainsonine[119, 120] and tyrosine betaine.[121] Modulation of histone acetylation provides an additional avenue to map the parvome of this economically important HEF biopesticide. Meromusides and similar acyl isocoumarins are likely biosynthesized by hrPKS-nrPKS collaborating systems in HEF where the nrPKS accepts a reduced starter unit (a triketide for meromusides) assembled by the hrPKS partner; extends it with additional ketide units (four of these for meromusides); and catalyzes the formation of the isocoumarin ring system using its PT and TE domains.



Eight new acyl isocoumarin glycosides (**93-100**) were also isolated from the solid culture of another *M. robertsii* strain (No. DTH12-10). Compound **93** exhibited strong antibacterial activity against *Pseudomonas aeruginosa* by inhibiting its quorum sensing system, reducing biofilm formation and the secretion of the virulence factors pyocyanine and rhamnolipids.[122]



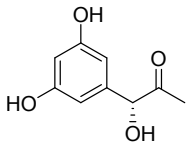
Four known isocoumarins, cytogenin (**101**), peyroisocoumarin D (**102**), diaportinol (**103**), and (+)-mucoricoumarin C (**104**) were isolated from the crude extract of cultures of the wasp-pathogen *Ophiocordyceps sphecocephala* BCC 2661.[123]



A series of isocoumarins were isolated from various strains of the scale insect pathogen *Conoideocrella tenuis*. These included 6,8-dihydroxy-3-methylisocoumarin (**106**), 6,8-dihydroxy-3-hydroxymethylisocoumarin (**109**) and the isocoumarin glucosides **105**, **107**, and **108** from strain BCC 12732;[72] the isocoumarin glycoside (**110**) from BCC 18627;[71] and the new (**111-115**) and known (**116-119**) isocoumarin analogues from BCC 44534.[61] These compounds may derive from a collaborative hrPKS-nrPKS system where the hrPKS partner is lost, nonfunctional or at least not competent for chain extension, hence the nrPKS utilizes a simple acetate unit for chain initiation. Compounds **105-109** were evaluated in antimalarial, antimycobacterial, antiviral, and cytotoxicity assays against KB, MCF-7, NCI-H187, and Vero cells. However, only compound **109** was found to moderately inhibit the growth of both Herpes simplex virus-1 and *Mycobacterium tuberculosis* H37Ra. Although the crude extracts from BCC 44534 displayed antimalarial activity against *Plasmodium falciparum* K1 (IC_{50} 9.53 μg/mL) and cytotoxic activity against a human small-cell lung cancer cell line (NCI-H187, IC_{50} of 4.60 μg/mL), the isolated compounds did not show such activities, with the exception of compound **111** that was weakly toxic to NCI-H187 and Vero cells (IC_{50} of 27.7 μg/mL and 9.6 μg/mL, respectively; c.f. ellipticine as the positive control with IC_{50} of 4.0 and 1.9 μg/mL, respectively).[61]

3.1.4. Other polyketides

Two phenolic compounds (**120** and **121**) were isolated from the scale insect pathogen *Conoideocrella tenuis* BCC 44534.[61] Neither compounds showed antimalarial, antibacterial, or cytotoxic activities. Another new phenolic polyketide, annullatin E (**122**), was obtained from the Lepidoptera pathogen *Isaria tenuipes*.[39] Diphenyl ethers **123-124** that may derive from the nonreduced polyketide orsellinic acid, together with another polyketide (**125**), were discovered as a result of the deletion of histone acetyltransferase (HAT) gene in *M. robertsii*.[29]



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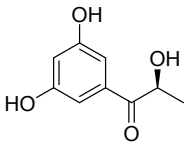

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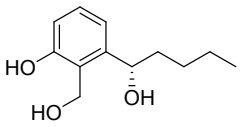
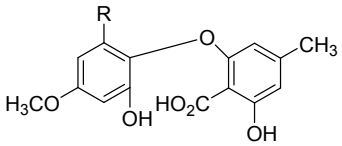
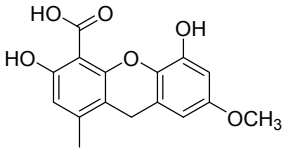


Annullatin E (**122**)



R = CH_2OH (**123**)
R = COOH (**124**)



125

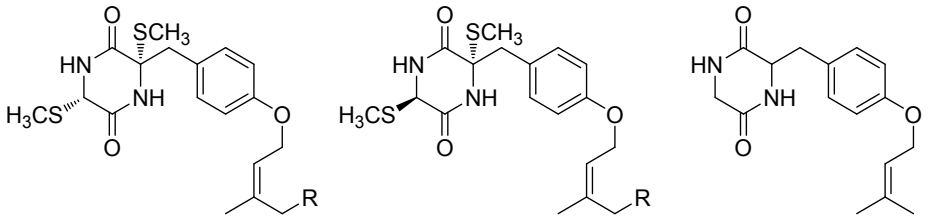
3.2. Nonribosomal peptides

Fungal nonribosomal peptides (NRPs) are synthesized by modular nonribosomal peptide synthetase (NRPS) enzymes. Typical NRPS modules feature an adenylation (A) domain that selects and activates an amino acid, hydroxycarboxylic acid or ketocarboxylic acid monomer; a thiolation (T, also known as a peptidyl carrier protein or PCP) domain that covalently tethers the incoming monomer or the growing NRP chain; and a condensation (C) domain that catalyzes the addition of the incoming monomer to the growing NRP chain. Additional processing domains may modify the newly added monomer and include *N*-methyltransferase (NMT), epimerase (E) or oxidase (Ox) domains, while variant C domains may yield heterocycles by intramolecular cyclization (Cy). Release of the full-length NRP product is achieved

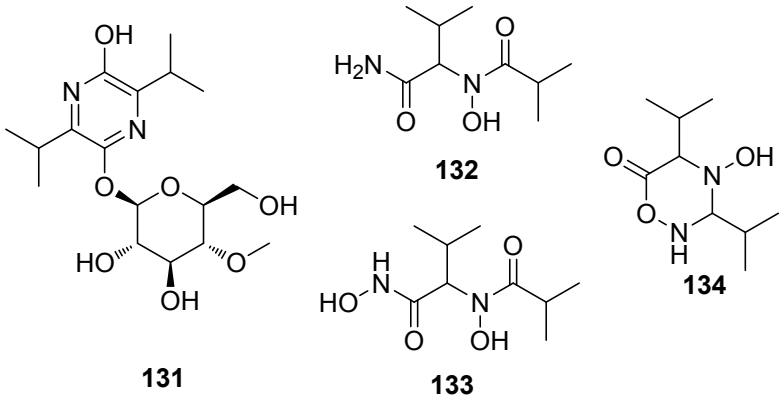
by a terminal C, a TE or an R (reductive) domain to afford cyclic, linear, or branched peptide chain topologies. NRPs may also incorporate carboxylic acid or fatty acid moieties attached to amino acid residues. These often derive from common amino acids, such as α-hydroxyisocaproic acid from Leu.[124] However, medium- and long-chain linear or branched fatty acids are often provided by dedicated fatty acid synthase (FAS) or PKS partner enzymes.[125, 126] Detailed information on the structural and functional aspects of these domains has been reviewed.[65, 128, 129]

3.2.1. Cyclic nonribosomal (depsi)peptides

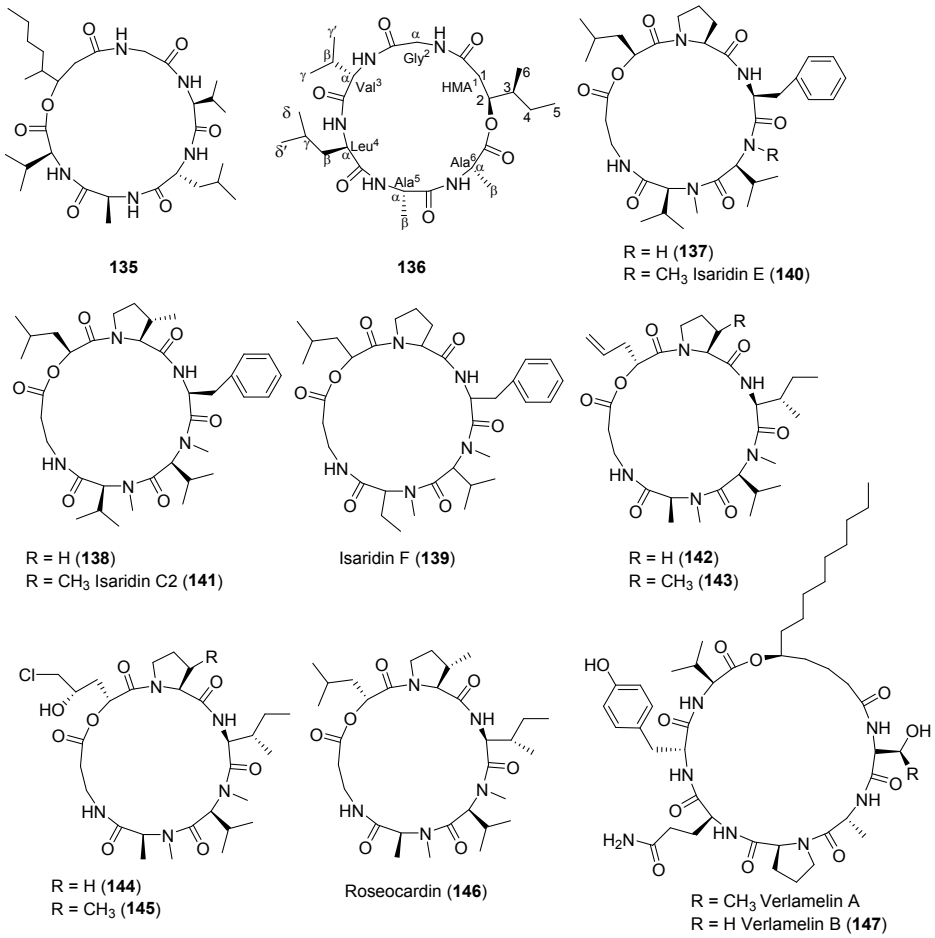
Three *O*-prenylated cyclic dipeptides, the meromutides A (**126**), B (**127**) and **130** were discovered in cultures of a *M. robertsii* strain with a deletion of the histone acetyltransferase (HAT) gene.[29] Meromutides A and B are the hydroxylated derivatives of the known compounds Sch 54794 (**128**) and Sch 54796 (**129**), respectively.[127]



The glycosylated pyrazine, akanthozine (**131**), and three other compounds (the hydroxamic acids **132** and **133** and the oxadiazinanone congener **134**) with undefined configuration(s) at the chiral center(s) were discovered in the culture of the spider-associated fungus, *Hevansia* (formerly *Akanthomyces) novoguineensis*.[59] Other pyrazines have been reported to possess a broad range of bioactivities.[128, 129] However, compounds **131-134** did not show antimicrobial activities against *Bacillus subtilis* DSM10, *Escherichia coli* DSM498, *Candida tenuis* MUCL29892 and *Mucor plumbeus* MUCL49355, nor did they display cytotoxicity against KB-3-1 (human cervix carcinoma) and L929 (established mouse fibroblast) cells.[59]



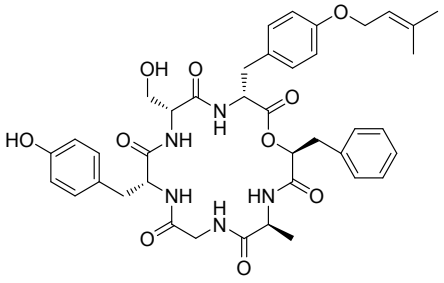
Five new cyclodepsipeptides, iso-isariin B (**135**) and D (**136**), desmethylisaridin E (**137**), desmethylisaridin C2 (**138**), and isaridin F (**139**), together with seven known cyclodepsipeptides, isaridin E (**140**)[130] isaridin C2 (**141**), destruxin A (**142**), roseotoxin B (**143**), destruxin E chlorohydrin (**144**), [β-Me-Pro] destruxin E chlorohydrin (**145**) and roseocardin (**146**) were isolated from *Beauveria felina* EN-135,[64, 130] *B. felina* KMM 4639[103] and *B. felina* BCRC 32873 in the presence of the histone deacetylase inhibitor suberoylanilide hydroxamic acid.[131] Compound **139** features a rare α-*N*-methylbutyric acid constituent. Iso-isariin B (**135**) caused significant lethality against the insect pest *Sitophilus* spp. with an LD_{50} value of 10 μg/mL, indicating that iso-isariin may act as a virulence factor of *B. felina*.[130] The hexadepsipeptides **136** and **142-145** exhibited potent toxicity against the brine shrimp (*Artemia salina*), with LD_{50} values of 26.58, 5.34, 0.73, 2.16, and 1.03 μM, respectively. These values are notably lower than that of the positive control colchicine (LD_{50} of 88.4 μM).[64] Using anti-inflammatory activity assays with human neutrophils, compounds **137-139** and **141** were shown to suppress FMLP-induced superoxide anion generation, while compounds **138**, **139**, **141**, and **146** inhibited the release of elastase. Importantly, these compounds exhibited no toxicity to human neutrophils.[131]



Another cyclic hexadepsipeptide, verlamelin B (**147**) was isolated from *Lecanicillium* sp. HF627.[132] Compound **147** is a new analogue of verlamelin, a NRP antibiotic originally isolated from *Simplicillium* (formerly known as *Verticillium*) *lamellicola*. Verlamelin B displays weak *in vitro* antifungal activity against plant pathogenic fungi such as *Magnaporthe grisea*, *Bipolaris maydis* (formerly known as *Cochliobolus heterostrophus*) and *Botrytis cinerea*. Compound **147** causes morphological changes to fungal cells such as swelling or bulging, while *in vivo* it displays strong plant protective and curative activities, particularly against rice blast (*Magnaporthe grisea*) and barley powdery mildew (*Erysiphe graminis* f.sp. hordei).[133, 134] The macrocycle of verlamelins is closed by an ester bond that forms between a secondary alcohol of the fatty acyl starter unit and the carboxyl group of the terminal Val.

A new cyclohexadepsipeptide, conoideocrellide A (**148**) and its linear derivatives,

conoideocrellides B-D (**149-151**) were obtained from the scale insect pathogenic fungus *Conoideocrella tenuis* BCC 18627.[71]



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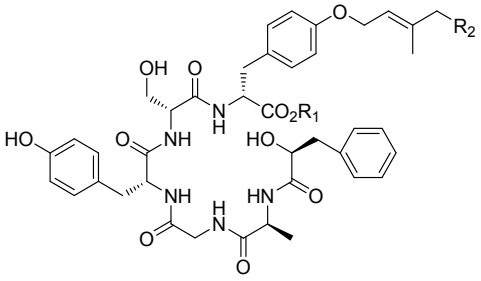
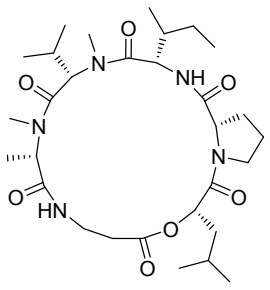


R_1 = H, R_2 = H (**149**)
R_1 = CH_3, R_2 = H (**150**)
R_1 = CH_3, R_2 = OH (**151**)

Three known depsipeptides, destruxin A (**142**), destruxin B (**152**) and destruxin E chlorohydrin (**153**), together with the known nortriterpenoid, helvolic acid (**154**) were isolated from a pigment-deficient mutant strain of *Ophiocordyceps coccidiicola* NBRC 100683.[135] These compounds have previously been detected in *Metarhizium* spp. including *M. anisopliae*.[112, 117] Compounds **142** and **152**–**154** showed strong *in vitro* anti-trypanosomal activity against *Trypanosoma brucei brucei* GUTat3.1 with IC_{50} values of 0.33, 0.16, 0.061 and 5.08 μg/mL, respectively (c.f. suramin as the positive control with an IC_{50} of 1.58 μg/mL). Compound **154** also displayed antibacterial activities, just as its analogue fusidic acid.[136] Human African trypanosomiasis, also known as sleeping sickness, is a potentially fatal parasitic disease transmitted by the bite of the tsetse fly that plagues many regions of Africa.



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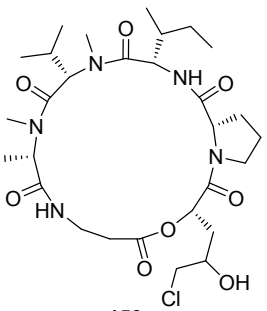

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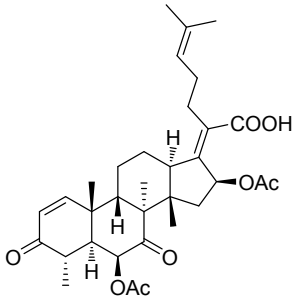
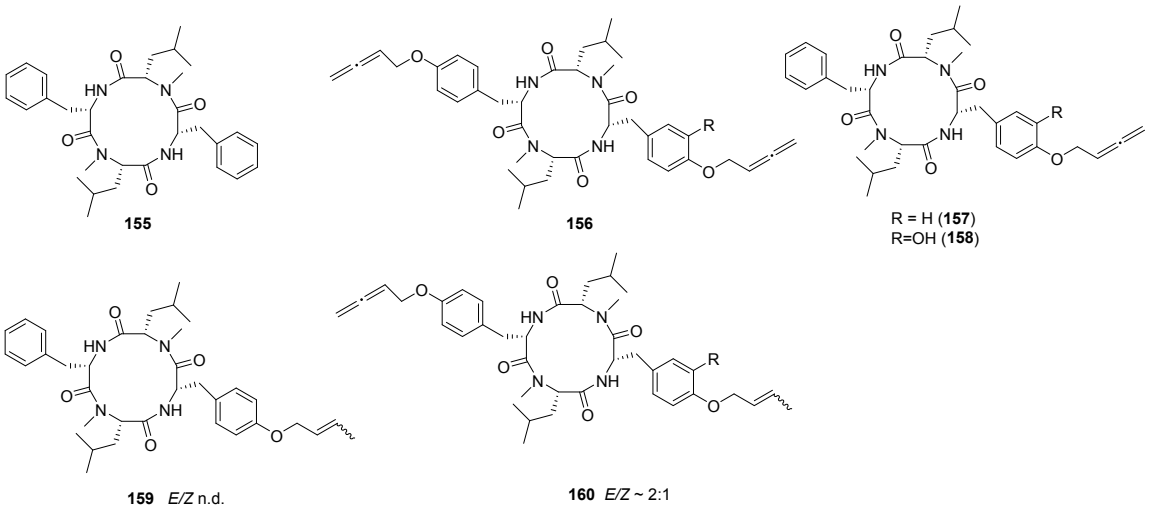


154

3.2.2. Cyclicoligomer (depsi)peptides

Cyclooligomer (depsi)peptides are biosynthesized by iterative NRPS enzymes that assemble several copies of an oligopeptide intermediate, then catalyze the recursive condensation and final cyclization of these monomers. Six new cyclotetrapeptide compounds, pseudoxylallemycins A-F (**155-160**) were obtained from the culture of the termite-associated HEF *Pseudoxylaria* sp. X802 when elicited by co-culturing with *Coriolopsis* sp.[137] Compounds **156-158** possess a rare allenyl moiety. Compounds similar to pseudoxyallemycins have been reported in the spider-derived HEF *Hirsutella* sp.,[3] in the saprobiont *Onychocola sclerotica*,[138] and in a mangrove-endophytic *Xylaria* sp.[139] *Pseudoxylaria* sp. X802 showed strong antifungal activities during co-cultivation with *Termitomyces* spp., and weak to moderate antifungal activities when grown together with other fungi such as *Cladosporium perangustum*, an unidentified Pleosporales sp., *Alternaria* sp., *Trichoderma* (formerly *Hypocrea*) *virens*, and *Fusarium* sp. However, none of the isolated compounds exhibited antifungal properties. Instead, compounds **155−158** displayed antibiotic activity against the human pathogenic bacteria *Pseudomonas aeruginosa* and *Mycobacterium vaccae*; showed antiproliferative activity against human umbilical vein endothelial and K-562 (human immortalized myelogenous leukemia) cells; and exhibited cytotoxic activity against HeLa cells.[137]



3.3. Polyketide–nonribosomal peptide hybrid metabolites

BGCs encoding PKS-NRPS hybrid enzymes are widely distributed in filamentous fungi.[143] A typical PKS-NRPS hybrid enzyme consists of a single module of an iterative PKS followed by a single module of an NRPS. These hybrid synthetases generate enormous SM structure diversity by combining the programmatic versatility of polyketide biosynthesis with the substrate flexibility of NRPS modules that are able to incorporate more than 300 proteinogenic and nonproteinogenic amino acids.[140-142]

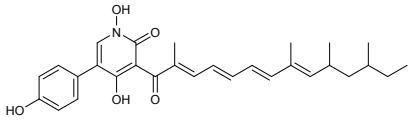
3.3.1. Pyridones

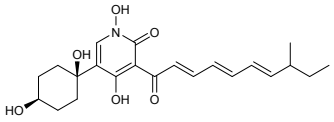
Pyridones are frequently encountered amongst the SMs of HEF,[65] exemplified by the cytotoxic militarinone D from *Cordyceps* (formerly *Paecilomyces*) *militaris*,[3] the neuritogenic (+)-*N*-deoxymilitarinone A from *Cordyceps farinosa* (formerly *Paecilomyces farinosus*),[143] the antibiotic ilicicolins from *Cylindrocladium ilicicola*,[144] the pigments tenellin and bassianin,[143, 145] the anti-allergic pyridovericin[146] and the protein tyrosine kinase inhibitor pyridomacrolidin from *B. bassiana*.[3] The structures and bioactivities of, and synthetic approaches towards 2-pyridones produced by HEF have been reviewed previously.[2, 3]

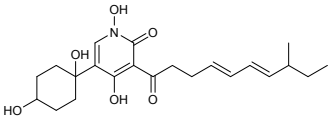
Fumosorinone (**161**) is a pyridine alkaloid obtained from *Isaria fumosorosea*.[147] Its structure is similar to tenellin and desmethylbassianin but differs from those in its acyl chain length and degree of methylation. Fumosorinone is a classic noncompetitive inhibitor of protein tyrosine phosphatase 1B (PTP1B, IC_{50} of 14.04 μM), a negative regulator of insulin receptor signaling and a potential drug target for the treatment of type II diabetes and other associated metabolic syndromes. Fumosorinone causes an increase in insulin-provoked glucose uptake and a decrease in the expression of PTP1B in insulin-resistant HepG2 cells, and activates the insulin signaling pathway.[147] The BGC of fumosorinone was validated through gene knockout using *Agrobacterium*-mediated transformation, and includes genes for a hybrid polyketide synthase-nonribosomal peptide synthetase (PKS-NRPS), two

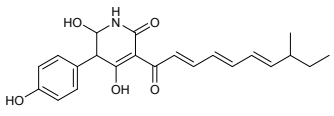
cytochrome P450 monooxygenases, a *trans* enoyl reductase, and two transcriptional regulators.

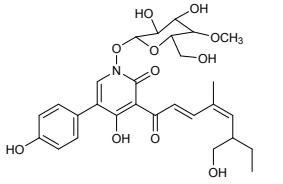
Three new pyridone alkaloids, JBIR-130 (**162**), JBIR-131 (**163**) and JBIR-132 (**164**), were discovered from *Isaria* sp. NBRC 104353.[148] Compounds **162** and **163** displayed no significant cytotoxicity against A549 cells (IC_{50} of 87 and 53 μM, respectively).[148] At sub-IC_{50} concentrations, **162** arrested the cell cycle at the G1 phase without evidence of direct toxicity.[148]


Fumosorinone (**161**)


JBIR-130 (**162**)


JBIR-131 (**163**)


JBIR-132 (**164**)

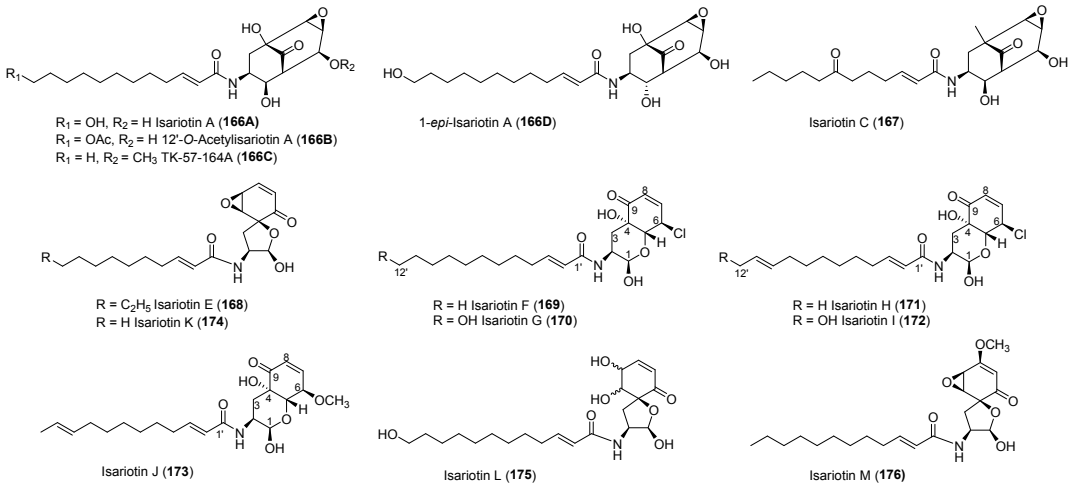

Pyridovericin-*N*-*O*-glucopyranoside (**165**)

The 2-pyridone alkaloid, pyridovericin and its new 4-*O*-methyl-*β*-d-glucopyranoside derivative, pyridovericin-*N*-*O*-glucopyranoside (**165**) as well as pretenellin B were isolated from *Beauveria bassiana* mycelia cultured in rice-oat medium.[65] Pyridovericin is derived from a reduced and *C*-methylated pentaketide condensed to a Tyr.[3]

3.3.2. Isariotin alkaloids

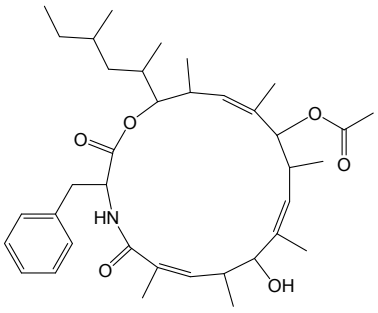
In addition to the known cytotoxic alkaloids isariotins A–F (**166-169**),[3, 149, 150] isariotins G-J (**170-173**) were reported from liquid fermentations of *Cordyceps* (formerly *Isaria* or *Paecilomyces*) *tenuipes*, a HEF that infects the pupae or larvae of lepidopteran insects.[151] Isariotins G-J display antimalarial activity against *Plasmodium falciparum* K1 and cytotoxic activity against KB, MCF-7, NCI-H187, and Vero cells.[151] Supplementation of chemical epigenetic modulators SBHA (a histone deacetylase inhibitor) and RG-108 (a DNA methyltransferase inhibitor) to *Gibellula formosana* cultures led to the isolation of five new isariotin analogs, 12'-*O*-acetylisariotin A (**166B**), 1-*epi*-isariotin A (**166D**), and isariotins K-M

(**174-176**), alongside previously isolated isariotins A (**166A**), C (**167**), E (**168**) and TK-57-164A (**166C**).[44] The total synthesis of isariotins E (**168**), F (**169**) and TK-57-164A (**166C**) has been reported,[152] but the biosynthetic pathway for these compounds remains undiscovered. While the bicyclo[3.3.1]nonane ring in isariotins A–D, and the spirocyclic or bicyclic hemiacetals of isariotins E-M, respectively, may be derived from tyrosine or phenylalanine, the unsaturated fatty acyl chain is likely of fatty acid or PK origin.[3]

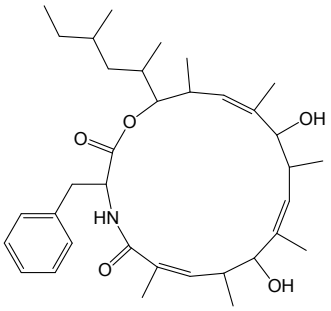


3.3.3. Macrocyclic PK-NRP hybrids

Two new macrocyclic PK-NRP hybrid molecules consisting of a Phe unit condensed with a nonaketide, the metacridamides A (**177**) and B (**178**) were isolated from the conidia of the HEF *Metarhizium acridum*. Metacridamide A showed moderate toxicity against Caco-2 (epithelial colorectal adenocarcinoma), MCF-7 (breast cancer) and HepG2/C3A (hepatoma) cells with IC_{50} of 6.2, 11.0, and 10.8 μM, respectively, while metacridamide B was only weakly active against HepG2/C3A cells (IC_{50} of 18.2 μM).[153] Neither compounds were found to exhibit insecticidal, antimicrobial, larvicidal, or phytotoxic activities.[153]



Metacridamide A (**177**)

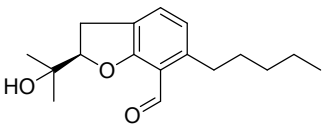


Metacridamide B (**178**)

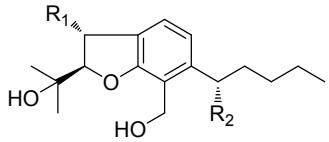
3.4. Terpenoids and polyketide-terpene meroterpenoids

3.4.1. Benzofuran meroterpenoids

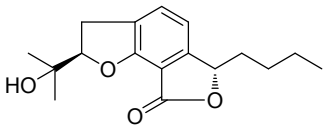
Compounds with benzofuran or benzofuranone moieties are rarely encountered from HEF. Four new 2,3-dihydrobenzofurans, annullatins A–D (**179–183**), were isolated from *Cordyceps annulata* when cultured in medium supplemented with the histone deacetylase inhibitor, SBHA.[39] Annullatins feature a benzene ring acylated with a C5 unit, akin to that of tetrahydrocannabinol (THC). Correspondingly, compound **179** was observed to possess strong agonistic activity toward the cannabinoid receptors CB1 and CB2; **180** exhibited CB1 agonistic activity; and **182** showed CB2 inverse agonistic activity.[39] Although earlier reports disclosed a few related benzofurans from filamentous fungi that have a C3 unit attached to the aromatic ring,[154, 155] this is the first demonstration that these alkylated 2,3-dihydrobenzofurans may act as ligands for cannabinoid receptors.



Annullatin A (**179**)



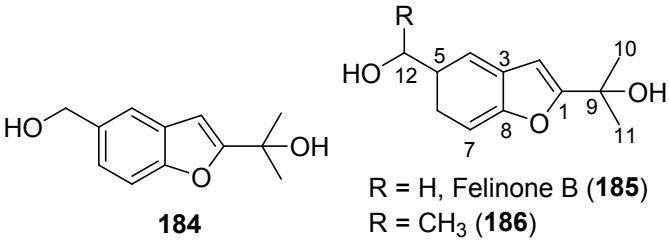
R_1=R_2=H Annullatin B (**180**)
R_1=OH, R_2=H Annullatin C (**181**)
R_1=H, R_2=OH (**183**)



Annullatin D (**182**)

Another two new benzofuranes, **184** and felinone B (**185**) were obtained from the HEF *Beauveria* (formerly *Isaria*) *felina* KMM 4639 and *B. felina* EN-135 respectively.[64, 102] The structure of **185** was very similar to that of **186**, a compound previously isolated from the eudicot plant *Smallanthus fruticosus*.[64] Felinone B (**185**)

exhibited weak toxicity against the brine shrimp with a lethal rate of 59.6% at a concentration of 100 μg/mL, and showed an antibiotic effect against *Pseudomonas aeruginosa* with an MIC of 32 μg/mL (compared to 4 μg/mL of the chloramphenicol control).[64]

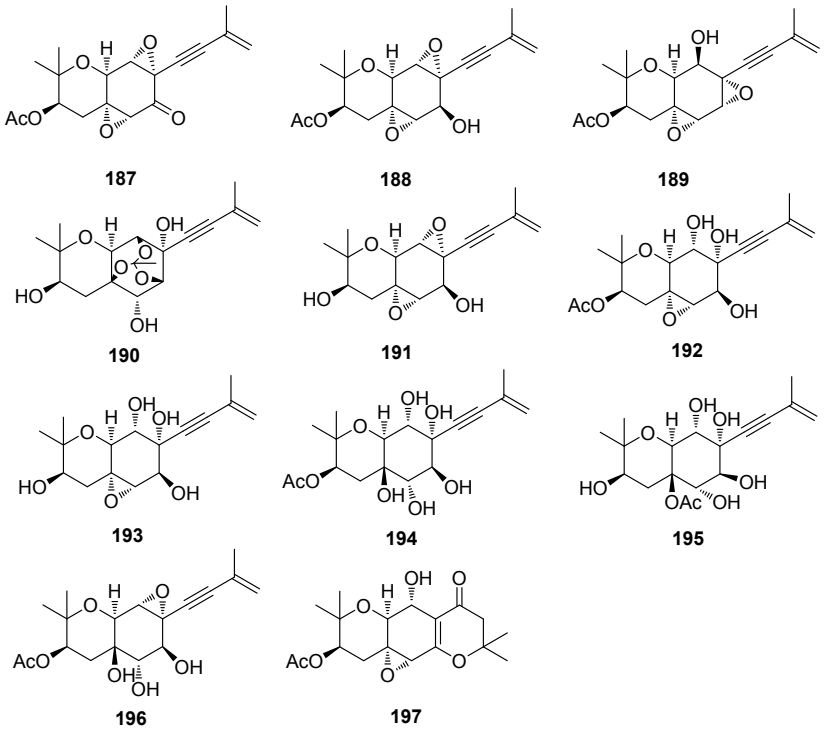


184

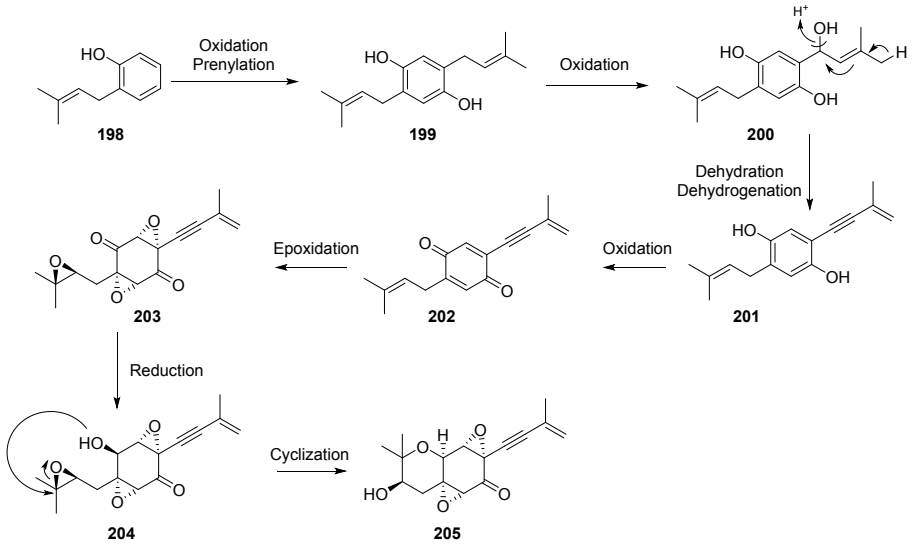
R = H, Felinone B (**185**)
R = CH_3 (**186**)

3.4.2. Chromene meroterpenoids

Ten new highly oxygenated chromene derivatives, oxirapentyns B-K (**188-197**) and the known oxirapentyn A (**187**)[156] were obtained from a lipophilic extract of a marine isolate of the HEF *Beauveria* (formerly *Isaria*) *felina* KMM 4639.[62, 63, 102] Oxirapentyn A (**188**) showed toxicity against SK-Mel-5 and SK-Mel-28 (human malignant melanoma), and T-47D (human breast cancer) cells with IC_{50} of 25, 19 and 17 μM, respectively (c.f. cisplatin as the positive control with IC_{50} of 252, 140 and 360 μM, respectively). No toxicity was detected against the CD-I mouse splenocytes.[54] Oxirapentyns A (**188**) and D (**190**) showed weak bacteriostatic activity against *Staphylococcus aureus* and *Bacillus subtilis* with MICs of 150μM and 140 μM, respectively.[54] Oxirapentyns G-K displayed no antibiotic activity against *S. aureus* ATCC 21027, *Bacillus subtilis* KMM 430, *Escherichia coli* ATCC 15034, *Pseudomonas aeruginosa* KMM 433, and *Candida albicans* KMM455. Oxirapentyn E at low and ultralow (10-10^{-12} μM) doses stimulated the growth of rootlets from corn (*Zea mays*) and barley (*Hordeum vulgare*).[55]



The biosynthetic pathway leading to the oxirapentyns may start with a prenylated phenolic precursor such as **198** (Scheme 3)[157] that may undergo oxidation and another prenylation to generate the diprenylated hydroquinone **199**. Another oxidation may yield the trihydroxy intermediate **200**. The subsequent formation of the methyl butenynyl moiety of **201** may be catalyzed by a dehydratase yielding a *Z*-double bond,[158, 159] followed by dehydrogenation by an acetylenase.[102, 160] Oxidation of **201** may afford quinone **202** that would undergo three epoxidation events to form intermediate **203**. After another reduction, intramolecular cyclization of **204** may yield the pyran **205** and establish the oxirapentyn framework.[102, 161] Further reductions and oxidations would then generate the different oxirapentyn analogues.

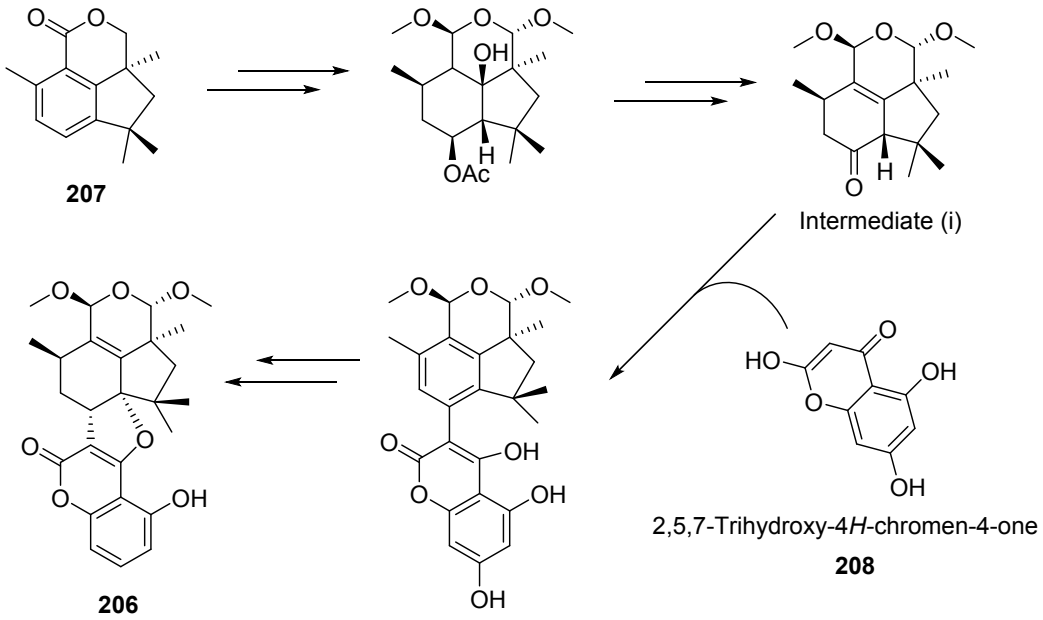


Scheme 3. Proposed pathway for the biosynthesis of oxirapentyns. Adapted from Yurchenko *et al.*[102] with permission (Copyright 2014, American Chemical Society).

3.4.3. Botryanes

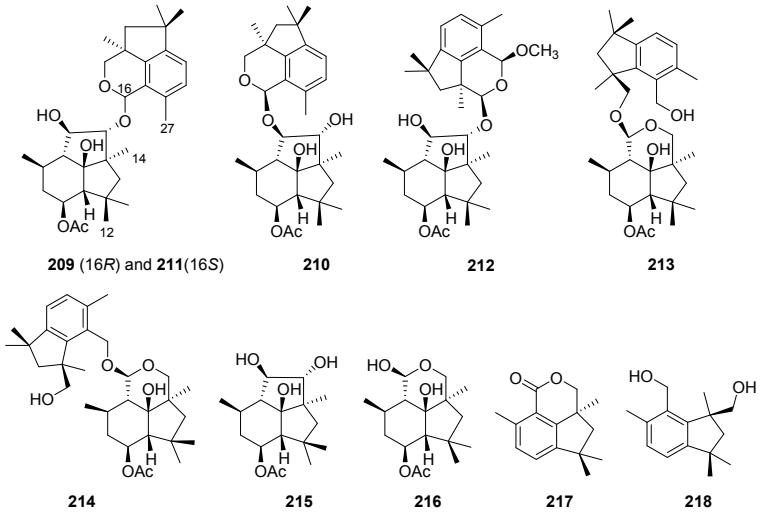
Botryane sesquiterpenoids have been encountered only from a limited number of fungal species.[162] Botryanes include the phytotoxin botrydial from *Botrytis cinerea*[163] and several antimicrobial botryanes from *Geniculosporium* sp.[164] A new botryane meroterpenoid with a unique hexacyclic 5/6/6/5/6/6 ring system, hypocrolide A (**206**) was isolated from the HEF *Trichoderma* (formerly *Hypocrea*) sp.[162163] Hypocrolide A was found to be weakly toxic to HeLa, A549, and MCF-7 cells with IC_{50} of 11.8, 22.0, and 20.4 μM, respectively (c.f. cisplatin as the positive control with IC_{50} of 4.7, 7.8, and 4.9 μM, respectively). Hypocrolide A (**206**) may be derived from a putative dihydrobotrydiol-like compound (intermediate i) and a coumarin-type precursor (**208**) by convergent biosynthesis (Scheme 4).[162] Intermediate (i) may originate from the known compound, 10-oxodehydrodihydrobotrydial (**207**)[165] that was also isolated from the hypcrolide A-producer *Trichoderma* sp. in this work.[162] The putative 2,5,7-trihydroxy-4*H*-chromen-4-one precursor (**208**) was not isolated, although several metabolites with this moiety were detected from the same *Trichoderma* sp.,

including 2,8-dihydroxy-3-methyl-9-oxoxanthene-1-carboxylic acid methyl ester,[166] and microsphaeropsones B and C.[167]



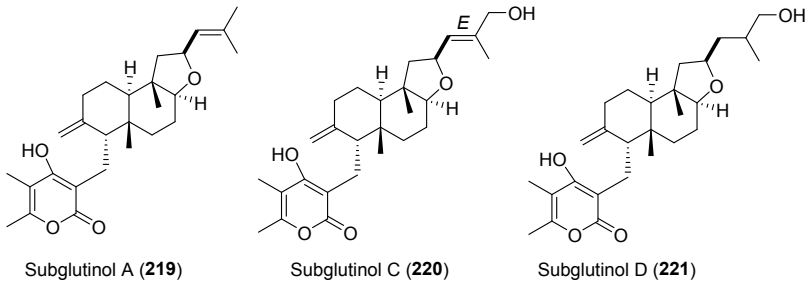
Scheme 4. Outline of the proposed biosynthesis of hypocrolide A (206). Adapted from Yuan *et al*.[162] with permission (Copyright 2013, American Chemical Society).

Six additional botryane meroterpenoid ethers, named hypocriols A−F (**209**−**214**) and four known botryanes, 4*β*-acetoxy-9*β*,10*β*,15*α*-trihydroxyprobotrydial (**215**), 10-oxodehydro-dihydrobotrydial (**216**), 10-oxodehydrodihydrobotrydial (**217**), and dehydrobotrydienol (**218**) were also discovered in the culture extract of *Trichoderma* (formerly *Hypocrea*) sp. EC1-35. Hypocriols A-F showed cytotoxic activities against HeLa, HCT116 (human colorectal carcinoma), A549, and MCF-7 human cancer cells.[168]



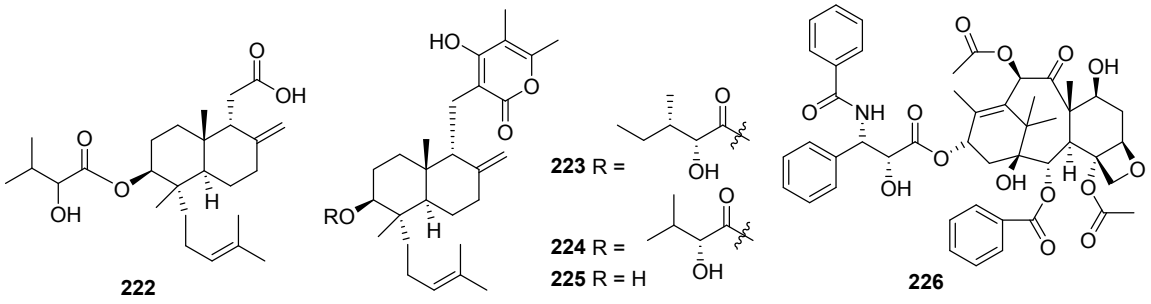
3.4.4. Diterpene meroterpenoids

Two new meroterpenoids, subglutinol C (**220**) and D (**221**), were isolated from *Metarhizium robertsii* ARSEF 23, alongside the known compound subglutinol A (**219**).[55] Subglutinols feature a PK-derived α-pyrone (4-hydroxy-5,6-dimethyl-2-pyrone) moiety attached to a diterpene-derived ring system consisting of a decalin core fused to a five-membered cyclic ether with a prenyl side chain. Subglutinol A was first isolated from the hypocrealean fungus, *Fusarium subglutinans* (teleomorph: *Gibberella fujikuroi*) and is known to be an immunosupressor.[169]



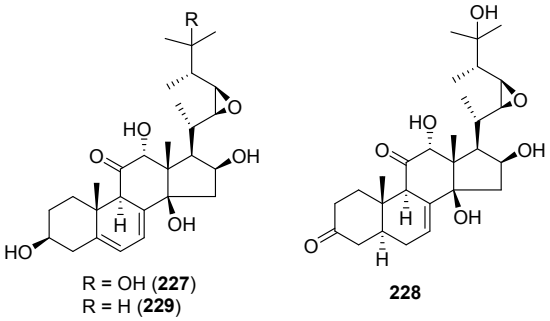
A new diterpenoid, conoideocin A (**222**), and three known pyrone diterpene meroterpenoids, metarhizins A (**223**) and B (**224**)[170], BR-050 (**225**),[171] were isolated from *Conoideocrella tenuis* BCC 44534.[61] Conoideocrellide A exhibited a broad range but weak biological activities including antimalarial (*Plasmodium falciparum*,

IC_{50} of 6.6 μg/mL, c.f. mefloquine hydrochloride as the positive control with an IC_{50} of 0.02 μg/mL), antibacterial (*B. cereus*, MIC of 25 μg/mL, c.f. vancomycin hydrochloride as the positive control with an IC_{50} of 2.0 μg/mL) and cytotoxic activities (KB and NCI-H187 cells, IC_{50} of 25.8 and 47.4 μg/mL, respectively, c.f. doxorubicin hydrochloride as the positive control with IC_{50} of 0.5 and 0.1 μg/mL, respectively). Compounds **223-225** were reported to exhibit antimalarial and antiproliferative activities against both insect cells and human cancer cells.[3, 170, 171]



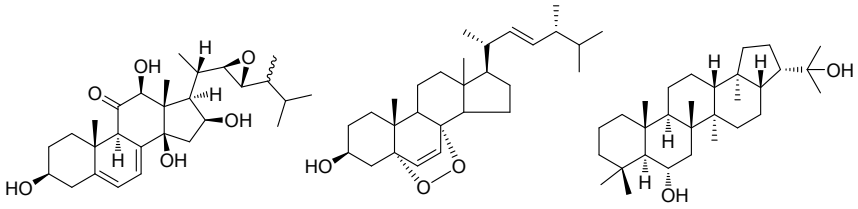
3.4.5. Steroidal and hopane triterpenoids

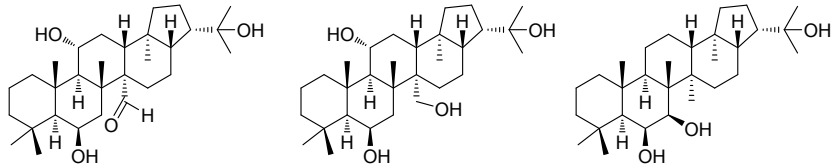
Two new highly oxidized ergosterols, formosterols A (**227**) and B (**228**) as well as the known compound formosterol C (**229**) were isolated from *Gibellula formosana*.[44] Formosterol C was previously isolated from a HEF fungus belonging to the *Isaria* genus.[44] The side chain of formosterols A-C features a *cis*-22,23-epoxide motif that is rare in naturally occurring sterols and triterpenes.



A new steroidal compound, JBIR-14 (**230**), was obtained from the culture extract of *Isaria* sp. NBRC 104353 while searching for inhibitors of the dynactin-associated protein, a new cancer target.[172] The epoxysteroid

5α,8α-epidioxy(22*E*,24*R*)-ergosta-6,22-dien-3*β*-ol (**231**)[173] and zeorin (**232**)[3] were isolated from *Aschersonia luteola* BCC 31749.[80] **231** exhibited antibacterial activity against *Bacillus cereus* and toxicity against NCI-H187 and Vero cells.[172]



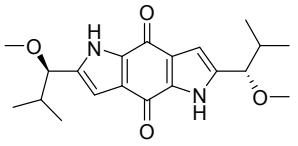


Zeorin (**232**)[174] and hopane-6b,7b,22-triol[71] were also isolated from the scale insect pathogen *Conoideocrella luteorostrata* Zimm BCC 31648, while three new hopane triterpenoids (**233-235**) and zeorin (**232**) were obtained from *Conoideocrella tenuis* BCC 18627. Compound **230** showed antiviral activity against type 1 herpes simplex virus (HSV-1, IC_{50} of 21 μM, comparable to acyclovir as the positive control with an IC_{50} of 17 μM), weak cytotoxicity against KB cells (IC_{50} of 10 μM, c.f. doxorubicin hydrochloride as the positive control with an IC_{50} of 0.27 μM), and weak to negligible activity against MCF-7 (IC_{50} of 28 μM), NCI-H187 (IC_{50} of 68 μM) and Vero cells (IC_{50} of 69 μM). Compound **231** exhibited weak antimalarial activity against *Plasmodium falciparum* K1 (IC_{50} of 9.8 μM, c.f. dihydroartemisinin as the positive control with an IC_{50} of 0.0044 μM), strong antiviral activity (IC_{50} of 14 μM) and weak cytotoxic activities against KB (IC_{50} of 5.6 μM) and MCF-7 cells (IC_{50} of 15 μM, c.f. doxorubicin hydrochloride as the positive control with an IC_{50} of 4.9 μM).[71]

3.5. Miscellaneous secondary metabolites

The dipyrrolobenzoquinone terreusinone A (**236**) was isolated from *Cordyceps gracilioides*.[84] The structure of **236** was similar to terreusinone, a UV-A protecting

compound from a marine alga-associated strain of *Aspergillus terreus*, a human opportunistic pathogen.[175] Terreusinone A inhibited the protein tyrosine phosphatases CDC25B and SHP1 at 4.1 and 5.6 μM, respectively.[84]



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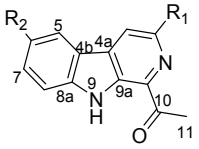
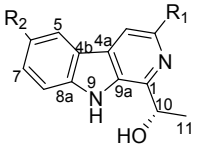
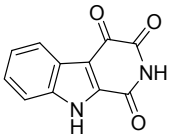


R_1 = OCH_3, R_2 = H (**240**)
R_1 = R_2 = OH (**241**)
R_1 = OH, R_2 = OCH_3 (**242**)
R_1 = OH, R_2 = H (**243**)
R_1 = R_2 = H (**244**)
R_1 = $COOCH_3$, R_2 = H (**245**)



R_1 = OH, R_2 = H (**237**)
R_1 = $COOCH_3$, R_2 = OH (**238**)
R_1 = $COOCH_3$, R_2 = H (**239**)



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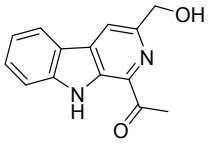
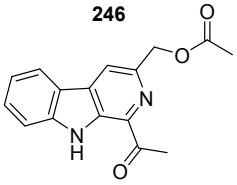
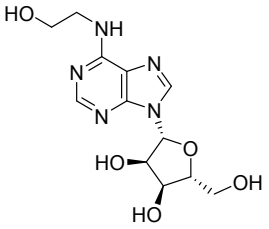


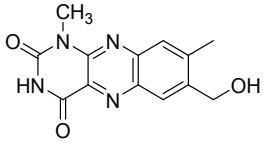
Gibellamine A (**247**)



Gibellamine B (**248**)



N6-(2-Hydroxyethyl)-adenosine (**249**)



1-Methyl-11-hydroxylumichrome (**250**)

A total of ten *β*-carboline alkaloids **237–246** were isolated from cultures of the wasp pathogen *Ophiocordyceps sphecocephala* BCC 2661. The compounds include five new *β*-carbolines, sphecolines A (**237**), B (**238**), and D–F (**240–242**); two *β*-carbolines, sphecolines C (**239**) and G (**243**) that are new to nature; and three known ones, 1-acetyl-*β*-carboline (**244**),[176] 1-acetyl-3-carbomethoxy-*β*-carboline (**245**),[177] and 1,3,4-trioxo-1,2,3,4-tetrahydo-*β*-carboline (**246**).[178] Two new *β*-carbolines, gibellamines A and B (**247** and **248**) were identified from extracts of cultures of *Gibellula gamsii* BCC47868 that had been isolated as a parasite of

unidentified small spiders.[179] *β*-carbolines are a large group of indole alkaloids with a tricyclic pyrido[3,4-b]indole ring structure that have been described as SM constituents of plants, marine invertebrates, and fungi. They show a wide range of biological activities, including antimicrobial, antiviral, antitumor, antimalarial, and hallucinogenic activities. Sphecolines A (**237**) and C (**239**) exhibited negligible cytotoxic activity against NCI-H187 cells, with IC_{50} of 79.9 and 75.1 μM, respectively (c.f. doxorubicin as the positive control with an IC_{50} of 0.13 μM).[123] 1-Acetyl-*β*-carboline (**244**) had previously been isolated from a marine actinomycete and exhibited antibiotic activity against various bacterial strains.[176] 1-Acetyl-3-carbomethoxy-*β*-carboline (**245**) had also been isolated from the eudicot plant *Vestia lycioides*[177] and showed cytotoxic activity against HCT116 cells.[180] Gibellamine A (**247**) inhibited biofilm formation of *Staphylococcus aureus*, while gibellamine B (**248**) exhibited weak cytotoxicity against A549, L929 and A431 (human squamous carcinoma) cells with IC_{50} values of 13, 16 and 24 μg/mL, respectively.[179] The biosynthesis of the *β*-carboline core was proposed to involve a Pictet-Spengler (PS) reaction. Enzymes known to mediate PS cyclization have been described to be involved in saframycin biosynthesis in *Streptomyces lavendulae*[181] and marinacarboline biosynthesis in *Marinactinospora thermotolerans*.[182]

The nucleoside N^6-(2-Hydroxyethyl)-adenosine (**249**) is a calcium antagonist that exhibits various bioactivities including insecticidal activities, inhibition of tumor cell proliferation, protection of kidney function, and prevention of inflammation. Its production was reported in several *Cordyceps sensu lato* species, and later in strains of *Beauveria bassiana*.[183]

Two alloxazines, the novel analogue 1-methyl-11-hydroxylumichrome (**250**), along with lumichrome, a well-known riboflavin (vitamin B_2) derivative, were isolated from *Beauveria bassiana* mycelia cultured in rice-oat medium.[63] Lumichrome had previously been described as a signaling molecule produced by the bacterium *Sinorhizobium meliloti* that enhances root respiration in alfalfa (*Medicago sativa*) and triggers a compensatory increase in whole-plant net carbon assimilation at a dosage as

low as 3 nM.[187] Neither of the two lumichrome congeners exhibited cytotoxic activity against cancer cell lines.[63]

4. Activities of HEF SMs related to insect pathogenicity

Important progress has been achieved in elucidating the biological roles of isolated SMs in the survival and the infection/parasitic cycle of HEF. These findings emphasized the importance of studying the intrinsic functions of SMs not solely to better understand the mechanisms of the fungus-host interactions, but also to facilitate the development of lead compounds for medical and agricultural applications.[28]

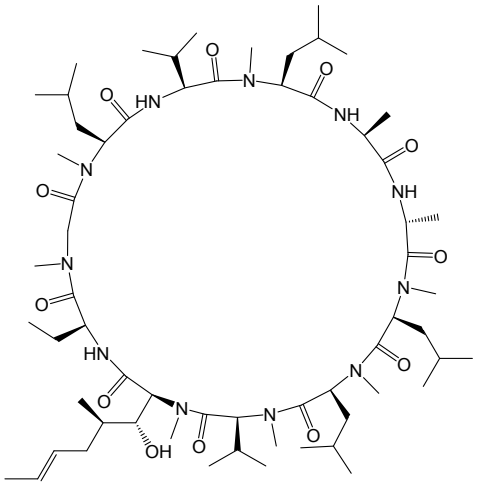
Cyclosporine A (CsA, **251**) is among the most well-known fungal secondary metabolites. It is a cyclo-undecapeptide originally described as a SM with a narrow spectrum of antifungal activities from the entomopathogenic fungus *Tolypocladium inflatum*.[184] CsA was subsequently recognized to act as a highly potent immunosuppressant,[185] and developed into a crucial medication used after organ transplantation, and to treat immunological disorders. However, the physiological importance of CsA for the producer fungus itself remained largely unknown until recently when strains with deletions in the CsA BGC were used to establish that this NRP is required for the full virulence of the fungus.[22] During insect mycosis, CsA hinders the immune responses of the host by targeting lipophorins, regulators of both humoral and cellular immune responses in insects.[186] CsA also moderately decreases lysozyme production in the insect haemolymph and severely suppresses their production of antibacterial peptides.[187] This immune-suppressed state of the host presents an opening for the invading entomopathogenic fungus, but may also cause dysbiosis in the commensal microbiome of the host and the overgrowth of opportunistic pathogens that may contribute to insect mortality. In addition, CsA also inhibits P-glycoprotein-related ATP-dependent efflux pumps in insects, thus this SM also reduces the ability of the host to exclude other mycotoxins produced by the entomopathogen from its cells.[187] As the mycosis progresses, CsA accumulation in the host may also help *T. inflatum* to secure the carcass and outcompete other,

opportunistic fungi as a result of the antifungal activity of this SM.[22] Details of the biosynthesis of CsA in *T. inflatum* have also been clarified recently.[22]

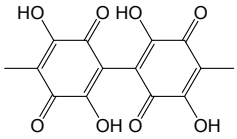
Oosporein (**252**) is a red, symmetrical 1,4-bibenzoquinone PK pigment that was first identified from *Beauveria bassiana* in the 1960s.[3, 188, 189] Oosporein production has also been reported in *B. brongniartii*,[190] *Phlebia* sp. (Basidiomycetes),[191] and various other phytopathogenic and endophytic fungi.[192-195] Oosporein displays various bioactivities, including insecticidal, antibiotic, antiviral, and antifungal activities.[3, 21] As a mycotoxin, oosporein causes gout in chickens and turkeys, and in larger doses, oosporein toxicosis may even be lethal to birds.[196] This raises concerns about the safety of the application of *Beauveria* spp. as biopesticides.[189]

The physiological role of oosporein in entomopathogenesis remains somewhat controversial. Recent reports indicate that oosporein promotes the infection process not by causing direct insecticidal outcomes, but by contributing to the ability of HEF to overcome the immune responses of the host insect.[21, 197, 198] Oosporein was seen to inhibit the prophenoloxidase activity of the host and repress the expression of antifungal peptides such as gallerimycin in the larvae of the greater wax moth,[21] or defensin 1 and cecropin 1 in the mosquito *Anopheles stephensi*.[197] Oosporein was also demonstrated to inhibit the expression of the dual oxidase in the midgut of the mosquito,[197] thereby reducing the capacity of the insect to produce reactive oxygen species. These immune response suppressing effects suggest that oosporein may facilitate the establishment of the fungal infection. Thus, oosporein-producing *Beauveria bassiana* strains germinated and escaped haemocyte encapsulation faster than nonproducer mutants.[21] In addition, the same effects also suggest that oosporein may contribute to the profound consequences of *B. bassiana* mycosis on the insect gut microbiome: Infection of mosquitoes by this HEF was shown to reduce bacterial diversity while increasing total bacterial load in the midgut. In particular, overgrowth of *Serratia marcescens* in the midgut of mosquitoes led to the translocation of these commensal bacteria into the haemocoel where they became opportunistic pathogens,

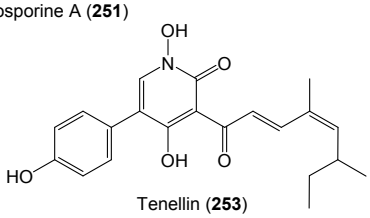
accelerating the death of the insect via sepsis.[197] Oosporein itself was shown to increase the density of yeast cells in the larvae of the greater wax moth (*Galleria mellonella*) and those of the large pine weevil (*Hylobius abietis*) upon infection with *Candida albicans*.[198] However, another report demonstrated that the expression of the oosporein PKS was only induced after the death of the *Galleria mellonella* host, but not during *B. bassiana* attachment, penetration, immune evasion and invasion of the host tissues.[199] Oosporein production was similarly only detected after the death of the host. Accumulation of this SM was seen to correlate with a dramatic decrease of the bacterial load in the insect cadaver, in accord with the known antimicrobial activities of oosporein.[192, 199] These observations suggest that the primary role of this SM in *B. bassiana* is the suppression of microbial competitors in the insect cadaver and the safeguarding of the dead host as a nutrient source for the fungus.[199] Further experiments will undoubtedly clarify the role of oosporein (as opposed to the many other SM products of *B. bassiana* and similar HEF) in the early stages of insect infection, and will provide a unifying and time-resolved picture of the dynamic contributions of this SM to fungal virulence, interactions with competing microorganisms, and insect mortality (c.f. Fan *et al.*[199] with Wei *et al*.[197] and Feng *et al.*[21]). Such investigations will necessitate the mapping of the multilevel regulatory circuits and their input signals that govern oosporein biosynthesis and harmonize the production of this metabolite with those of other SMs and additional, proteinaceous virulence factors in *B. bassiana*. To this effect, a novel zinc finger transcription factor (BbSmr1) that represses oosporein production was identified by T-DNA insertion mutagenesis in *B. bassiana.*[199] A feedback induction mechanism was also reported to regulate oosporein production in *Beauveria caledonica* where exogenous oosporein induced the expression of the oosporein BGC, and led to the increased abundance of the oosporein biosynthetic enzymes.[198]



Cyclosporine A (**251**)



Oosporein (**252**)



Tenellin (**253**)

Tenellin (**253**) and similar 2-pyridones are widely distributed in HEF. Nevertheless, mutants that produce no tenellin due to the knockout of key biosynthetic genes showed no loss of virulence in assays with *Galleria mellonella* as the target insect, indicating that these metabolites play no direct role in insect pathogenesis.[2, 3] Recently, the hydroxamic acid moiety of tenellin was discovered to act as an iron III chelator that may reduce oxidative stress induced by excess iron in *Beauveria bassiana* cells.[200] In iron replete conditions, ferricrocin, an intracellular siderophore is produced by many fungi including *B. bassiana* to sequester this metal. Deletion of the ferricrocin synthetase gene in *B. bassiana* resulted in a sizeable increase of tenellin biosynthesis and the accumulation of the iron–tenellin complex.[200] Thus, tenellin may still be important for iron homeostasis under certain physiological conditions in *B. bassiana*.

5. Conclusions

Hypocrealean entomopathogenic fungi are proficient producers of secondary metabolites that mediate various biotic and abiotic processes in the native niche of these organisms, including virulence against the insect hosts. These SMs thus

contribute to the utility of HEF as commercial biopesticides in the context of integrated pest management systems, and provide lead compounds for the development of chemical pesticides for crop protection. However, the various bioactivities of these SMs should also be carefully evaluated from the ecotoxicological and environmental safety viewpoints, including the safety of farmers and consumers of agricultural products. The production of bioactive SMs also led to the widespread use of certain HEF as nutraceuticals or traditional remedies, and prompted the modern pharmaceutical industry to repurpose some of these molecules as life-saving (and highly profitable) human medications. Continued efforts to mine the parvome of HEF, and to characterize the multifaceted bioactivities of these fascinating metabolites will allow us to better understand the host-pathogen arms race, develop more efficient and safer (bio)pesticides, and identify lead compounds for drug discovery and development.

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7. Conflicts of interest

I.M. has disclosed financial interests in Teva Pharmaceuticals Works Ltd., Hungary and the University of Debrecen (Hungary) which are unrelated to the subject of the research presented here. All other authors declare no conflicts of interest.

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