Selected Fungal Natural Products with Antimicrobial Properties

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Natural products (NPs) are a very rich source of antimicrobial drugs. They constitute more than two-thirds of clinically used antibiotics and half of anticancer drugs. Fungal natural products and their effects have been known to humankind for hundreds of years. Their later medicinal applications, followed by the discovery of the first class of antibiotics, penicillins and other drugs of fungal origin, such as peptidic natural products, terpenoids or polyketides, have altered the historically negative reputation of fungal “toxins”.

Keywords: antimicrobial properties ; antimicrobial resistance ; biosynthesis ; ergot alkaloids ; fungal metabolites ; natural products ; peptides ; polyketides ; terpenoids

1. Introduction

Natural products (NPs) are a very rich source of antimicrobial drugs. They constitute more than two-thirds of clinically used antibiotics and half of anticancer drugs [1]. Molecules biosynthesised by fungi are a diverse and useful group of NPs. Plant endophytic and pathogenic fungi produce many secondary metabolites that play important roles in virulence and competition against other microbes. Due to their broad-spectrum activity, some of these NPs can also exhibit high biocidal activity against human pathogenic microbes. In recent years, marine fungi have emerged as a novel source of fungal NPs and may be a potential game changer in drug discovery; however, marine fungi still constitute an underrepresented resource of diverse NPs.

Fungal NPs have an important place in human history. For instance, ergot alkaloids (EAs) which are produced by the filamentous fungi of the genus *Claviceps*, have been referenced in ancient historical texts. References to grain diseases have been found in the Bible, in the Old Testament (850–550 BC). In the Middle Ages, the first reported ergotism epidemic was recorded in 944–1000 AD when almost half the population of the Aquitaine region of France (about 60,000 people) died of ergot poisoning [2][3]. The gangrenous form of the disease (medically known under the name Ergotismus gangraenosus) was commonly known as ‘‘ergotism’’, ‘‘holy fire’’ or ‘‘St. Anthony’s fire’’. Symptoms include delirium, hallucinations, muscle spasms, convulsions and gangrene of the limbs. The gruesome history of ergots has overshadowed their beneficial medicinal properties. However, the use of ergots as medicinal compounds was first documented in 1582, as they were administered for ‘‘quickening childbirth’’. Further research and screening of ergot analogues for oxytocic drugs that stimulate uterine contractions to hasten childbirth resulted, in 1938, in the synthesis of lysergic acid diethylamide (LSD) **5** (**Figure 1**B), a hallucinogenic compound that has become infamous for its use as an illicit “recreational drug” [4]. Currently, EAs are the inspiration for numerous semi-synthetic derivatives, such as cabergoline **6** or ergotamine **8** (**Figure 1**B,D, respectively) that have been applied in a wide range of medicinal treatments, such as the treatment of migraines, Parkinson’s disease, reduction of tumour growth, and other lesser-known synergistic antimicrobial activities.

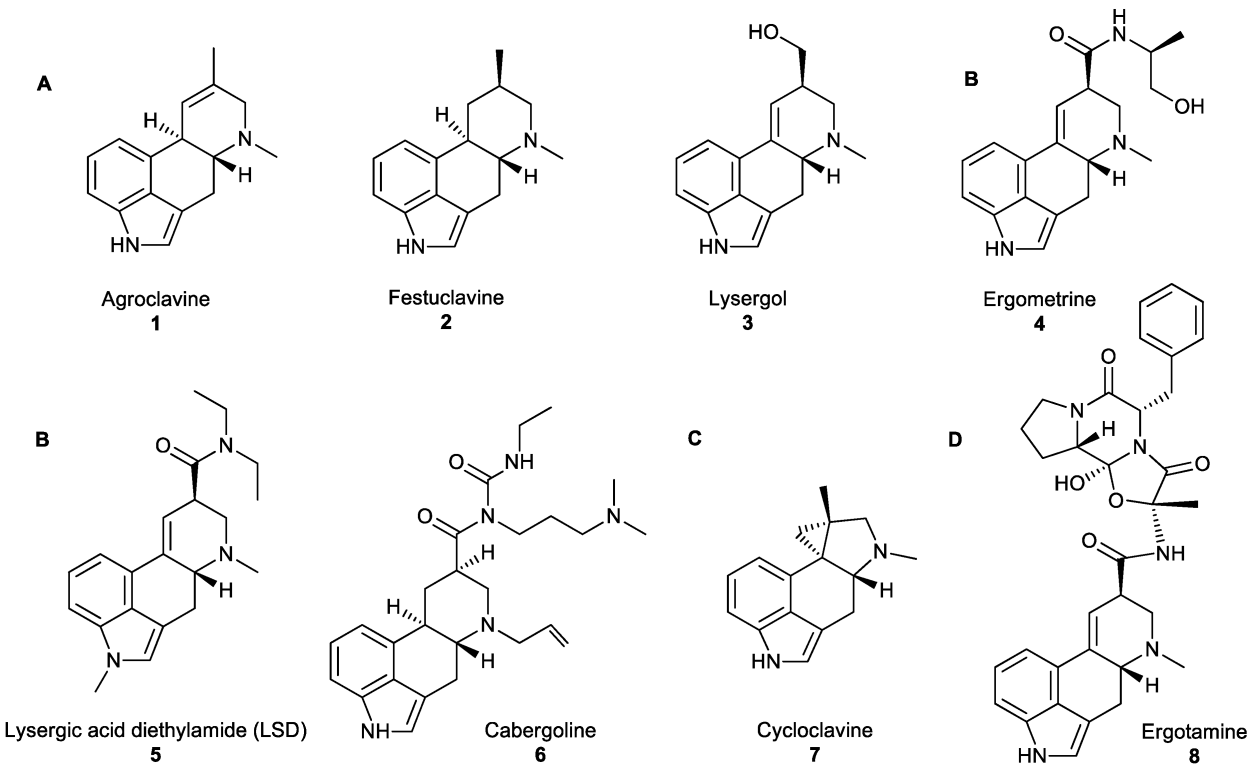

Figure 1. (**A**) Examples of clavines. (**B**) Simple lysergic acid derivatives. (**C**) The unusual ergoline scaffold of cycloclavine. (**D**) Ergopeptides consisting of D-lysergic acid with a cyclic tripeptide moiety [5].

2. Selected Examples of Antimicrobial Natural Products from Fungi

2.1. Ergot Alkaloids: Fungal Natural Products Derived from Amino Acids

All naturally occurring EAs share a common tetracyclic scaffold, the so-called ‘‘ergoline scaffold”, derived from L-tryptophan. EAs are divided into three major classes based on the substituents decorating this scaffold: clavines (festuclavine and agroclavine derivatives), simple lysergic acid derivatives and ergopeptides (**Figure 1**A,B,D, accordingly) [6]. Clavines include the partially or fully saturated ring species D, such as agroclavine **1**, festuclavine **2** or lysergol **3** (**Figure 1**A). Simple lysergic acid derivatives consist of the basic D-lysergic acid structure as an alkyl amide (**Figure 1**B), and ergopeptides also based on D-lysergic acid and a cyclic tripeptide moiety (**Figure 1**D). Cycloclavine **7** is a newly characterised ergot alkaloid which has been reproduced *in vitro*, and has an unusual ring system, where ring D has been transformed into a new five- and three-membered ring fusion [7].

EAs are produced by fungi occupying distinct ecological niches. *Clavicipitaceous* species, such as *Claviceps purpurea* and *Neotyphodium lolii* from the order *Eurotiales* are plant pathogenic and symbiotic fungi, respectively. *Aspergillus fumigatus* from the same order, *Eurotiales*, is an opportunistic pathogen of mammals which also produces EAs, such as festuclavine **2** [8][9]. Cycloclavine **7** is biosynthesised in nature by *Aspergillus japonicus*, which is frequently responsible for the post-harvest decay of fresh fruit (apples, pears, peaches, citrus, grapes, figs, strawberries, tomatoes or melons) and some vegetables (especially onions, garlic, and yams) [10].

The biosynthetic pathways of EAs have been well studied and are described in depth elsewhere [5][11]; however, a brief overview of EAs biosynthesis is given here. First, the prenylation of L-tryptophan by dimethylallyl pyrophosphate (DMAPP) yields 4-(γ,γ-dimethylallyl)tryptophan (DMAT) and is followed by the *N*-methylation of DMAT to 4-dimethyl-L-abrine (*N*-Me-DMAT). Subsequently, a series of successive oxidation steps catalyses the intramolecular cyclization of the prenyl and indole moieties to form ring C in tricyclic chanoclavine-I, which, in turn, is oxidised to form chanoclavine-I-aldehyde. At this branch point, chanoclavine-I-aldehyde undergoes intramolecular cyclization to form either ring D of tetracyclic agroclavine **1** (*C. purpurea, N. lolii*) or festuclavine **2** (*A. fumigatus*). Subsequently festuclavine **2** is further biotransformed into fumigaclavines. The new branch of this pathway is an unusual oxidation of the cyclic iminium form of chanoclavine-I-aldehyde catalysed by non-heme iron and α-ketoglutarate dependent oxidase EasH, to yield a unique cyclopropyl ring moiety which is fused to a five-membered ring, in cycloclavine **7**. The structure of EasH and possible mechanism of cycloclavine **7** formation has been published recently [12][13].

Ergot-derived medicines, such as ergometrine **4**, were used to facilitate obstetric deliveries or to treat postpartum haemorrhage. The high bioactivity of EAs is correlated with the ability of these compounds to act as agonists or antagonists toward neuroreceptors. Although some EAs, such as the hallucinogenic compound LSD **5** have been used as recreational drugs, most EAs were associated with medicinal applications, including treatments against migraine and tumour (ergotamine **8**), Parkinson's disease or restless leg syndrome (cabergoline **6**; **Figure 1**B). However, their synergistic antimicrobial activity is a less commonly known fact. Lysergol **3** is a synthetic EA that exhibits a synergistic antibiotic pharmaceutical activity as a bioactive enhancer and a bioavailability facilitator for broad-spectrum antibiotics. This property facilitates the absorption of antibiotics across the cell membrane in animal cells resulting in increased action against Gram-positive and -negative bacteria [6]. With the recommended dosage of lysergol of 10 µg/mL, the improved activity of antimicrobial effect is in the range of 2–12 folds, against a wide spectrum of both Gram-positive and -negative bacteria including *Escherichia. coli*, *Bacillus subtilis*, *Mycobacterium smegmatis* and other similar microorganisms.

2.2. Fungal Polyketides

The polyketide pathway constitutes one of the major biosynthetic pathways leading to the production of fungal NPs. Polyketides are polymers synthesised from simple carboxylic acid derivatives (e.g., acetyl-CoA, malonyl-CoA, and methylmalonyl-CoA) into linear chains by iterative Claisen condensation, followed, in some cases, by reductive modification of the resulting β-keto groups. These compounds are synthesised in fungi (and other organisms) by enzymes called polyketide synthases (PKSs). Polyketides are extremely diverse and include compounds such as polyesters, polyphenols, macrolides (macrocyclic esters), polyenes and enediynes.

Strobilurins are an important group of polyketide-derived fungal NPs which have yielded one of the major classes of fungicides currently in use to protect agricultural crops from fungal diseases. The discovery of these compounds occurred after the observation that *Strobilurus tenacellus* and *Oudemansiella mucida*, two agaricomycetes growing on decaying wood in European forests, were able to defend themselves against other fungi. Their antifungal activity was associated with the production of the compounds strobilurin A **9** and oudemansin A **10** in *S. tenacellus* and *O. mucida*, respectively (**Figure 2**) [14][15]. These two compounds inhibit the transfer of electrons between complexes II and III of the electron transport chain in the mitochondria, resulting in impaired cell respiration and ATP synthesis [16]. Despite strobilurin A **9** and oudemansin A **10** exhibiting high antifungal activity, these NPs are quickly degraded by light, rendering them unsuitable for use in crop protection. Many attempts were made to modify the chemical structures of natural strobilurins to increase photo-stability while maintaining antifungal activity [17]. After several years of research, azoxystrobin **11** was synthesised and became the first photo-stable strobilurin-derived active ingredient registered for use in crop protection (**Figure 2**) [18], paving the way for the synthesis of a multitude of fungicides belonging to the quinone outside inhibitor (QoI) class of fungicides. However, resistance to QoI fungicides arose after a few years of use in fields. The single point mutation which confers resistance to QoI leads to the substitution of the amino acid glycine for alanine at position 143 (G143A). This mutation is now widespread in many fungal species, including *Zymoseptoria tritici*, *Botrytis cinerea* and *Cercospora beticola*, the agents responsible for Septoria leaf blotch in wheat, Botrytis grey mould and Cercospora leaf spot in beets, respectively [19][20][21]. Despite numerous resistance issues, QoIs are still used to control some of the most devastating rust fungi, such as *Puccinia striiformis* and *Phakopsora pachyrhizi*, the causative agents of the yellow rust of cereals and soybean rust diseases, respectively [22].

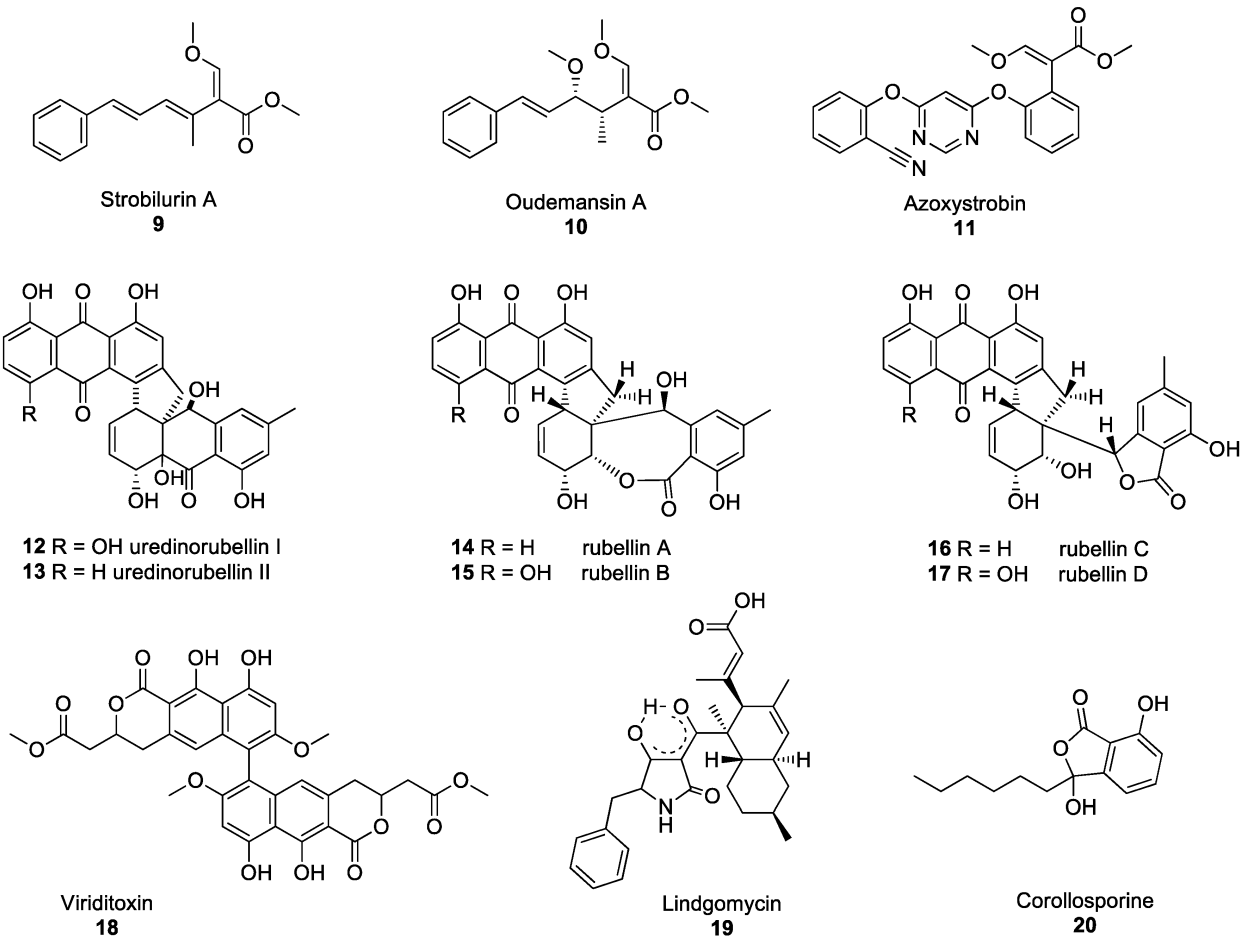

Figure 2. Structures of the selected fungal polyketides: Strobilurin A **9**, oudemansin A **10**, azoxystrobin **11**, uredinorubellins I and II (**12**–**13**) rubellins A–D (**14**–**17**), viriditoxin **18**, lindgomycin **19**, corollosporine **20**.

Some of the polyketide NPs synthesised by pathogenic fungi exhibit dimeric structures. Such is the case of lesser-known compounds produced by the *Torrubiella* species. The *Torrubiella* species are arthropod-pathogenic fungi that parasitise spiders, scale-insects and hoppers and are known to synthesise derivatives of uredinorubellin I **12** and II **13** (**Figure 2**). These compounds exhibited photodynamic activity, influencing cell viability in three mammalian cell lines, such as HIG82, HT29 and J774A.1, as well as antibacterial activity against *Staphylococcus aureus* [23]. The genus *Torrubiella* which belongs to the *Clavicipitaceae* family, is related to the genus *Ramularia* which also contains fungi that produce polyketide NPs with antimicrobial activity.

Ramularia collo-cygni is an ascomycete fungus responsible for the important plant disease Ramularia leaf spot (RLS) [24]. RLS is primarily a disease of barley but the fungus can infect other grain crops, such as wheat and oats as well as wild grasses. *R. collo-cygni* produces a range of secondary metabolites, including rubellin anthraquinones **14**–**17** (**Figure 2**). Rubellins are non-host-specific phytotoxins with photodynamic properties [25]. Miethbauer et al. showed that rubellins are biosynthesised via a polyketide pathway, by demonstrating the incorporation of both [1-^{13}C]-acetate and [2-^{13}C]-acetate into the rubellins during their formation [26]. McGrann and co-workers have recently sequenced and analysed the genome of *R. collo-cygni* and found that it contains the genetic architecture to synthesise a wide range of secondary metabolites, including rubellins [27]. In a later study, it was suggested that the co-expression of genes coding for PKSs and hybrid PKS/nonribosomal peptide synthetases (NRPSs) may be associated with the competitive colonisation of the host plant and early symptom development [28]. However, no exact determination of the biosynthetic pathway of rubellins has been elucidated yet and the role of these metabolites remains unclear. Despite the phytotoxic properties of rubellins, these compounds may have potential pharmaceutical applications. Miethbauer et al. have observed initial activities against Gram-positive bacteria, including MDR strains, such as *B. subtilis* (ATCC) 6633, *S. aureus* (SG) 511, *S. aureus* 134/94 (MRSA), *Enterococcus faecalis* 1528 (VRE) or *Mycobacterium vaccae* (IMET) 10670 [29]. Rubellins also exhibit antimicrobial, antiproliferative, cytotoxic and tau aggregation inhibitory activity *in vitro* tests [29]. Minimal inhibitory concentrations (MICs) were determined with and without illumination, showing a light-dependent increase in the antibacterial activity of compounds **14**–**17** (except against *M. vaccae*), with rubellin D **17** being the most active.

PKSs are not only involved in the biosynthesis of anthraquinone derivatives, such as rubellins, but also that of other dimers, such as viriditoxin **18** or lindgomycin **19** (**Figure 2**). Viriditoxin **18** belongs to the group of xanthoradones produced

by *Penicillium radicum* FKI-3765-2. Xanthoradones exhibit activity against methicillin-resistant *S. aureus* (MRSA) by inhibiting FtsZ, the bacterial tubulin homolog which is crucial in septum formation [30].

Lindgomycin **19**, an unusual antibiotic polyketide, contains two distinct structural domains, a bicyclic hydrocarbon and a tetramic acid that are connected by a carbonyl functional group. Naturally occurring tetramic acid derivatives, originating from a variety of marine and terrestrial fungi (Arctic fungus of the *Lindgomycetaceae* family), have attracted great interest due to the breadth of the spectrum of their biological activities, as well as their challenging structural complexity [31][32]. The majority of the compounds isolated to date have exhibited mostly antibiotic or antiviral activity. Lindgomycin **19** revealed good antibiotic activity against a number of Gram-positive bacteria (IC_{50}: 2–6 μM), as well as the yeast *Candida albicans* and the plant pathogenic fungus *Z. tritici* (IC_{50}: 5–10 μM) [33]. This compound also showed antibiotic activity against an MRSA strain with IC_{50} values of 5.1 μM. Another example of polyketide-derived NP from marine fungi is corollosporine **20** (**Figure 2**). Corollosporine **20** is an antibacterial phthalide derivative produced by the fungus *Corollospora maritima* which was isolated from driftwood found near the island of Heligoland, Germany [34].

2.3. Peptidic Fungal Natural Products

Two distinct pathways in fungi are responsible for the production of peptidic NPs. Enzymes in the nonribosomal peptide (NRP) pathway produce the majority of peptide metabolites. These are highly specific, multimodular enzymes called NRPSs, which utilise both proteinogenic and non-proteinogenic amino acids to synthesise the peptidic backbones. The genes encoding these enzymes are usually located within a biosynthetic cluster, comprising several genes that are co-regulated. The other pathway is that of ribosomally synthesised and post-translationally modified peptides (RiPPs); very large peptidic NPs with molecular weights typically around 1000 Da are synthesised through this pathway [35].

2.4. Terpenoid Compounds

Terpenoids are a large class of natural products related to terpenes, and are made of condensed isoprene units. Terpenoids are often considered mostly as plant NPs; however, fungal species are also known to produce terpene-derived metabolites. The screening of marine fungi for natural products with potential pharmaceutical applications has only recently begun, but has the potential to yield several new drugs.

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