

Natural Product Reports

Unlocking the Metabolic Potential of Endophytic Fungi Through Epigenetics: A Paradigm Shift for Natural Products Discovery and Plant-Microbe Interactions

Covering: up to December 2024

Rui Liu,^{a,#} Xiao-Ping Peng,^{a,#} David J. Newman,^b Diane Purchase,^c Gang Li^{*a} and Souvik Kusari^{*d}

^a*Department of Natural Medicinal Chemistry and Pharmacognosy, School of Pharmacy, Qingdao University, Qingdao 266071, People's Republic of China. E-mail: gang.li@qdu.edu.cn*

^b*Retired Chief, NCI Natural Products Branch, Wayne, PA 19087, USA*

^c*Department of Natural Sciences, Faculty of Science and Technology, Middlesex University, London NW4 4BT, UK*

^d*Pavol Jozef Šafárik University in Košice, Faculty of Science, Institute of Biology and Ecology, Department of Genetics, Mánesova 23, 04154 Košice, Slovakia. E-mail: souvik.kusari@upjs.sk*

[#]These authors contributed equally to this work.

^{*}Correspondence

Assoc. Prof. Dr. Gang Li (gang.li@qdu.edu.cn) <https://orcid.org/0000-0003-0621-2779>

Prof. Dr. Souvik Kusari (souvik.kusari@upjs.sk) <https://orcid.org/0000-0002-4685-0794>

Submitted 18 April 2025; Accepted: 17 July 2025; Available online: 4 Aug 2025

<https://doi.org/10.1039/d5np00028a>

Abstract

Microbial metabolic pathways, including those of endophytic fungi, offer significant potential for synthesizing secondary metabolites, regardless of their ecological niche. These pathways can be modulated at the molecular level through genome and epigenome manipulation. The metabolic activation of fungal endophytes using epigenetics presents an exciting frontier in science, paving the way for advanced biotechnological applications and enhancing our understanding of these microorganisms' roles in ecosystems. This review examines the significant role of epigenetics in the biosynthesis of secondary metabolites from fungal endophytes, which is vital for drug discovery. Our primary focus centers on studies that explore the epigenetic modulation of endophytic fungi up until December 2024. Acknowledging the rapidly evolving landscape of epigenetic research in this field, which has limited examples for endophytic fungi, we provide crucial foundational insights into fungal epigenetics and relate these insights to the broader context of plant–microbe interactions and endophytic fungal epigenetics, supported by relevant examples. Key mechanisms, such as histone acetylation, histone methylation, and DNA methylation, are discussed alongside recent advances in small-molecule epigenetic modulators that can activate silent biosynthetic gene clusters (BGCs). Further, chromatin-dependent regulation of these BGCs and methods for probing chromatin modifications and secondary metabolism in fungi are discussed. The role of CRISPR-Cas9 genome editing, combined with epigenetic strategies, is highlighted, showcasing its ability to alter the metabolite profiles of fungal endophytes. Finally, we explore how artificial intelligence (AI), machine learning (ML), and deep learning (DL) innovations are transforming research in chemical epigenomics at the plant–microbe interface.

1. Introduction
2. Biosynthetic Gene Clusters (BGCs) of Endophytic Fungi for Natural Product Biosynthesis
3. Chromatin-Dependent Regulation of Natural Product Biosynthesis in Fungal Endophytes
4. Methods for Probing Chromatin Modification and Secondary Metabolism in Fungi
5. Mechanistic Insights into Epigenetic Remodelling of Silent Gene Clusters
 - 5.1. Histone acetylation
 - 5.2 Histone methylation
 - 5.3 DNA methylation
6. Design and Synthesis of Small-Molecule Chemical Epigenetic Modulators
 - 6.1 “Cap-Linker-ZBG” type HDAC inhibitors
 - 6.1.1 Cap-based design and synthesis
 - 6.1.2 Linker-based design and synthesis
 - 6.1.3 ZBG-based design and synthesis
 - 6.2 Specific chemical scaffold-based epigenetic modifiers
 - 6.3 DNA-DBDs type for enhancing affinity
 - 6.4 Computer-driven analysis and design
7. Chemical Modulation of Endophytic Fungal Epigenetic Machinery
 - 7.1 HDAC inhibitors
 - 7.2 DNMT inhibitors
 - 7.3 The combination of HDAC and DNMT inhibitors
8. Inactivation or Overexpression of Epigenetic Regulators
 - 8.1 Erasers (HDACs)
 - 8.2 Writers (HATs and HKMTs)
 - 8.3 Readers (SntB)
 - 8.4 The integrated strategy
9. CRISPR/dCas9-Mediated Epigenetic Modification
10. Biological and Nature-Inspired Modulation of Endophytic Fungal Epigenetic Machinery
11. Epigenetic Modulation at the Plant-Fungal Interface
 - 11.1 DNA methylation
 - 11.2 Histone and chromatin modifications
 - 11.3 Other modulations
12. Artificial Intelligence (AI) in Epigenetic Modulation
13. Conclusions
14. Future Prospects
15. Data availability
16. Conflicts of interest
17. Acknowledgements
18. References

1. Introduction

Natural products featuring diverse chemical scaffolds and exhibiting significant biological activities remain an invaluable reservoir for bioactive small molecules.¹ Investigating these compounds from a wide range of natural organisms has been instrumental in advancing natural product-based drug discovery.² In recent decades, endophytic fungi (or fungal endophytes³) have become a critical source of bioactive secondary metabolites (Figure 1). These microorganisms inhabit the living tissues of healthy plants for at least a portion of their life cycle, typically without inducing visible disease symptoms.^{4,5} Since the early identification of endophytic fungi in the seeds of *Lolium temulentum* in the late 19th century,⁶ it has become widely recognized that virtually all plant species harbor at least one fungal endophyte, including those that are currently unculturable under laboratory conditions.⁷ These mutualistic endophytic microorganisms often enhance the metabolic functions of their host plants. However, disruptions in the signaling pathways maintaining these endophytic associations could result in pathogenic infections rather than beneficial mutualistic relationships.⁸ Additionally, these fungi confer protective advantages to the host plant against pests and diseases by producing bioactive compounds, such as defensive metabolites or allelochemicals.⁹ While our understanding of the selective pressures and regulatory mechanisms driving the synthesis of these biosynthetic compounds remains limited, the multitrophic interactions among plants, endophytes, and their associated organisms are believed to influence the metabolic landscapes of these microorganisms significantly.³

Given the vast biological diversity and the potential of plant-associated endophytic fungi to produce secondary metabolites, there is a strong motivation to utilize them as microbial factories for the biotechnological production of pharmaceutically relevant bioactive compounds (Figure 1). Surprisingly, until the late 20th century, these endophytes received insufficient attention from the natural product chemistry community. The ability of endophytic fungi to produce

bioactive compounds, particularly those effective against current and emerging diseases, holds significant ecological and industrial importance. Although their discovery may appear to be random, it stands to reason that, following extensive co-evolution, selective fungal endophytes could produce plant-derived natural products, their precursors, pathway intermediates, or structural analogs that possess therapeutic potential.¹⁰ Moreover, these fungi and their community demonstrate remarkable adaptability,¹¹ and can produce complex secondary metabolites that can function as prodrugs or therapeutic agents for various diseases.^{12,13} Their unique ability to biosynthesize diverse chemical structures makes them especially valuable in pharmaceutical research and development.

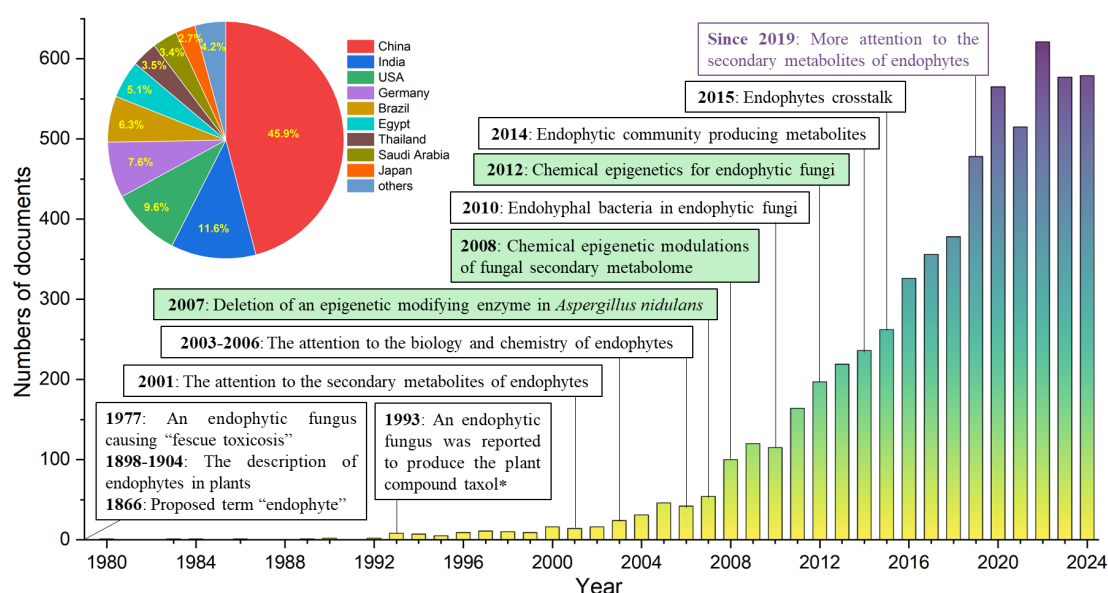


Figure 1. Summarization of the research on natural products from endophytic fungi. The histogram compares the number of publications regarding natural product research for endophytic fungi each year, while the pie chart compares the ratios of publications by country. Key research advances, including the epigenetic modifications of endophytic fungi, are indicated. The data were retrieved from the Web of Science Core Collection using keyword combinations: "endophytic fungus and compound" and "fungal endophyte and compound." The document type selected for the search was limited to research articles. The collected reference documents were randomly downloaded and checked manually to ensure the reliability of the data. *More discussions on the Taxol® biosynthesis by endophytic fungi can be found in section 10 in this review.

The secondary metabolites produced by fungal endophytes represent a small fraction of their biosynthetic potential.¹⁴⁻¹⁶ The challenge lies in the limited translation of discoveries involving endophytes capable of producing valuable compounds into industrial bioprocesses for the commercial production of biopharmaceuticals. This issue is often attributed to the silent or insufficient expression of relevant genes or the inability of isolated organisms to remain metabolically active in artificial environments.¹⁴⁻¹⁶ Epigenetic mechanisms, which are heritable and do not entail changes to the DNA sequence, serve as essential regulatory elements for fungi in modulating the transcription of biosynthetic genes.¹⁷⁻¹⁹ Focusing on epigenome-related histone and DNA post-translational modifications offers an opportunity to rationally induce the activation or suppression of biosynthetic gene clusters (BGCs) involved in secondary metabolite production.²⁰⁻²² This strategy may contribute to advanced biotechnological applications and improve our understanding of the roles of these microorganisms in ecosystems.

In this comprehensive review, we explore the pivotal role of epigenetics in enhancing the biosynthesis of secondary metabolites from fungal endophytes, which plays a significant part in the drug discovery process, encompassing studies reported up to December 2024. Given the limited number of studies specifically focused on endophytic fungal epigenetics, we draw upon broader examples from fungal epigenetics and plant-microbe interactions to provide essential context and foundational understanding. These examples are carefully selected to illustrate key mechanisms and concepts that are likely conserved or relevant in endophytic systems, thereby strengthening the conceptual framework and highlighting potential directions for future research in this emerging field. Our discussion extends to the recent advancements in synthesizing small-molecule epigenetic modulators and their capacity to activate silent or cryptic biosynthetic pathways through manipulating gene clusters. We also examine the application of the CRISPR-Cas9 genome editing

technology in conjunction with epigenetic interventions, highlighting its potential to reshape the metabolite profiles of fungal endophytes. Furthermore, we delve into the contributions of artificial intelligence (AI), machine learning (ML), and the latest developments in deep learning (DL) methodologies to chemical epigenomics at the plant-microbe interface, underscoring their transformative impact on research in this area.

2. Biosynthetic Gene Clusters (BGCs) of endophytic fungi for natural product biosynthesis

Endophytic fungi are increasingly recognized as having significant capabilities in natural product biosynthesis. The backbone, tailoring, and regulatory enzymes responsible for the biosynthesis of secondary metabolites are typically encoded by a cluster of co-located genes in the genome, which is known as a biosynthetic gene cluster (BGC).¹⁶ With the rapid growth of fungal genomic and metagenomic data, a significant number of fungal BGCs have been annotated. However, only a tiny fraction of these BGCs have been linked to the production of natural products identified to date, such as only 16% of the polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS)-derived *Penicillium* BGCs that could be connected to a characterized fungal pathway in the MIBiG database.²³ Filamentous fungi typically contain around 50 distinct BGCs per genome.²⁴ However, complex genetic and epigenetic regulatory networks in endophytic fungi often keep most of these BGCs cryptic or expressed at low levels under certain conditions, indicating that most endophytic fungal chemical diversity remains unexplored.²⁵ In particular, the substantial biosynthetic potential of endophytic fungi reflects the complex ecological environment, which is difficult to replicate in the laboratory. A representative example is the symbiosis between fungal endophytes in the *Epichloa* clade (*Epichloa* species and their asexual counterpart *Neotyphodium* species; Clavicipitaceae, Ascomycota) and grasses of the family Poaceae (sub-

family Poöideae).²⁶

Endophytic fungi in the *Epichloae* clade are capable of producing alkaloids of four distinct classes: ergot alkaloids (ergovaline), indole diterpenes (lolitrem B), 1-aminopyrrolizidines (loline), and pyrrolopyrazines (peramine) (Figure 2).²⁶ Loline and peramine alkaloids are deterrent and/or toxic to insects, while ergot alkaloids and indole diterpenes are two main classes of anti-mammalian alkaloids.^{27,28} Ergot alkaloids in endophyte-infected tall fescue caused “fescue toxicosis” in livestock, and indole-diterpene alkaloids in endophyte-infected ryegrass led to “ryegrass staggers”. Ergot alkaloids, such as ergovaline, are the most extensively researched, mainly because of their plentiful production and are thought to mainly influence vertebrate feeding deterrents.^{29,30} Today, ergot alkaloids act as templates for many semisynthetic derivatives used clinically to treat migraines, Parkinsonism, and tumor growth, with their diverse bioactivities associated with agonist/antagonist effects on dopamine, serotonin, and adrenaline neuroreceptors.

Ergot alkaloids belong to a family of indole derivatives characterized by a tetracyclic ergoline ring system.²⁹ They can be classified into three distinct subgroups: clavines, ergoamides, and ergopeptines. Specifically, clavine-type alkaloids, such as D-lysergic acid, simply consist of the tetracyclic ergoline ring system. In contrast, ergoamides and ergopeptines share the same ergoline ring system as the clavine-type alkaloids, but differ in their substituents: ergoamides are amides derived from D-lysergic acid, while ergopeptines are D-lysergic acid derivatives with tripeptide units attached to its carboxy group via amide bonds.²⁹ The tripeptide residues in ergopeptines always contain proline and form a tricyclic ring system. Specifically, ergovaline, the best-characterized ergot alkaloid produced by *Epichloë*, has a peptide moiety that includes alanine and valine, in addition to proline. In comparison, the phenylalanine in the well-known ergotamine produced by *Claviceps purpurea* is replaced by valine in ergovaline biosynthesized by grass endophytes.^{29,30}

The discovery of individual genes involved in biosynthetic pathways for each of the four alkaloid classes has resulted in the elucidation of their BGC, especially the well-documented BGC (*eas*) genes for ergot alkaloids. The ergot alkaloid biosynthesis genes within the endophytic *Epichloë* genomes have discontinuous distributions and show evident rearrangements of gene positions relative to each other and to the telomeres (Figure 2)^{31,32}. Additionally, the *eas* cluster, along with the indole diterpenes BGC (*ltm*), are highly expressed when the endophyte grows *in planta* and display a pattern of gene expression that classifies them as symbiosis-specific genes, indicating the vast metabolic potential harbored by endophytic fungi.³¹⁻³³ The *eas* gene cluster within the ergovaline-producing endophytic *E. festucae* contains 11 genes organized in three subclusters separated by large blocks of type I (retrotransposon) and type II transposon relics (Figure 2). The genes responsible for each enzymatic step in the ergovaline pathway have been identified by sequence similarity analyses, gene disruptions, genetic complementation, or *in vitro* biochemical characterizations.³⁴

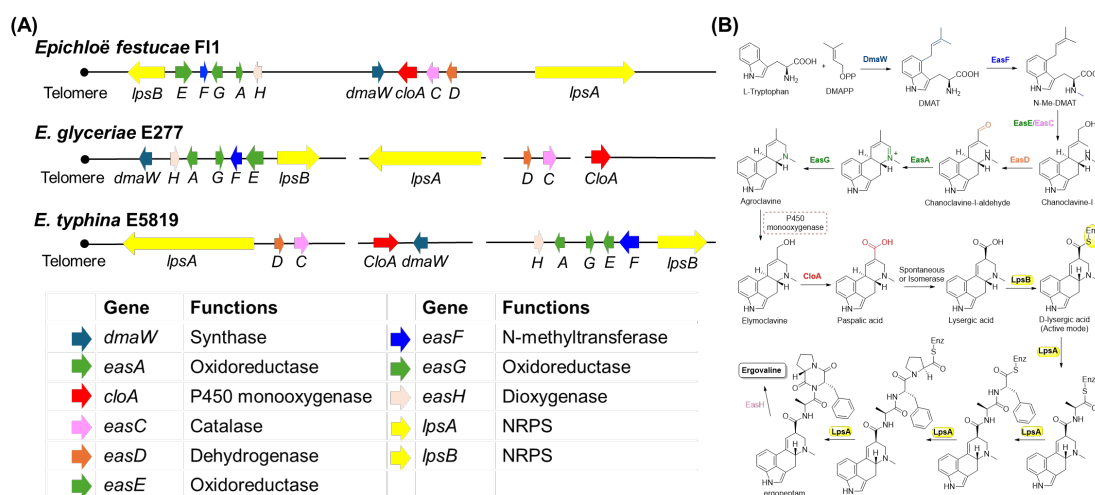


Figure 2. The BGCs of the representative ergot alkaloid, ergovaline, from endophytic fungi in the epichloae clade. (A) Physical map of the ergovaline (*eas*) biosynthetic gene clusters. The colored arrows identify the location of each cluster gene and the direction of transcription. Closed circles indicate telomeres. (B) The biosynthetic pathway of ergovaline.

The biosynthesis of all ergot alkaloids, including the grass endophytes-derived ergovaline, initiates with a pivotal first step (Figure 2): the formation of dimethylallyltryptophan (DMAT) from L-tryptophan and dimethylallyl diphosphate (DMAPP).^{29,30} This reaction is catalyzed by the dimethylallyltryptophan synthase (DMATS) DmaW.³⁵ As reported by Wang et al. in 2004, the disruption of the *dmaW* gene in endophytic *Neotyphodium* sp isolate Lp1 led to the complete loss of ergot alkaloids production.³⁶ The *easF* gene encodes an enzyme that catalyzes the N-methylation of DMAT in the presence of S-adenosyl methionine (SAM).^{29,37} Subsequently, following the action of the N-methyltransferase EasF, the EasC protein, which shows catalase activity, works in conjunction with EasE, a FAD-dependent oxidoreductase. Together, they are involved in the intramolecular cyclization of the prenyl and indole unit within N-Me-DMAT, resulting in the formation of tricyclic chanoclavine-I.^{38,39} The hydroxyl of chanoclavine-I is further oxidized to chanoclavine-I-aldehyde through EasD, a short-chain alcohol dehydrogenase.^{29,40} Chanoclavine-I-aldehyde serves as a common intermediate for all classes of ergot alkaloids. The EasA protein derived from the BGC of *N. lolii* is essential for the isomerization of chanoclavine-I-aldehyde and the formation of ring D in the cyclic iminium intermediate. This is followed by the reduction of a double bond by the NADPH-dependent oxidoreductase EasG, yielding agroclavine.⁴¹ Agroclavine is then converted, via the intermediate elymoclavine, to the ergolene carboxylic acid, paspalic acid, which spontaneously isomerizes to D-lysergic acid. In this conversion process, two enzymes with NADPH-dependent cytochrome P450 monooxygenase activities are required: agroclavine oxygenase and elymoclavine oxygenase (CloA).⁴² LpsA (LPS1) is a three-module non-ribosomal peptide synthetase (NRPS) and plays a crucial role in the formation of the tripeptide moiety in *N. lolii*.⁴³ LpsB (LPS2), on the other hand, is a single-module NRPS responsible for activating and linking lysergic acid in *N. lolii*.³³ It is proposed that lysergic acid is gradually

elongated to the mono-, di-, and tripeptides by the trimodular LpsA. The final hydroxylation at the aminoacyl residue, followed by a spontaneous heterocyclization step that leads to the production of the end product ergovaline, is proven to be catalyzed by a dioxygenase, EasH.⁴⁴

3. Chromatin-dependent regulation of natural product biosynthesis in fungal endophytes

The fungal genome is packaged into chromatin, a macromolecular complex comprising DNA, histones, and non-histone proteins. Chromatin can further be classified into heterochromatin and euchromatin (Figure 3), the two primary states of chromatin exhibiting significant differences in structure, function, and gene expression activity.⁴⁵ Heterochromatin is highly condensed and transcriptionally inactive, restricting proteins from accessing DNA and leading to gene silencing.^{46,47} In contrast, euchromatin is generally gene-rich, relatively relaxed, and less compacted. Despite consisting of condensed liquid-like domains, it allows easy access for DNA transcription machinery to the DNA, resulting in gene expression.⁴⁷ The interconversion from heterochromatin covering BGCs to euchromatin using chemical and genetic modulations of fungal epigenetic enzymes is promising for awakening BGCs.²⁰⁻²²

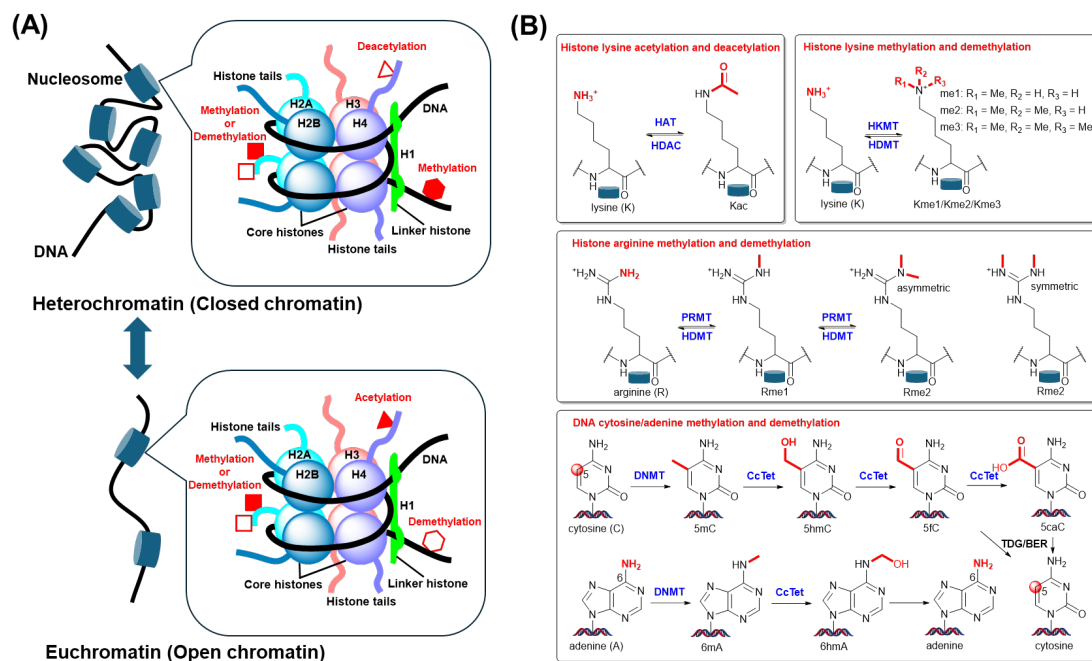


Figure 3. Epigenetic modifications. (A) The interconversion between heterochromatin and euchromatin, and the structure of a nucleosome; (B) Histone acetylation/deacetylation, histone methylation/demethylation, and DNA methylation/demethylation.

The basic and repeating unit of chromatin in eukaryotes is called a nucleosome, which consists of a segment of ~147 base pairs of DNA wrapped around an octamer of highly conserved core histones: two dimers of H3 and H4 as a central tetramer, together with two copies of H2A and H2B (Figure 3). Each core histone protein has an unstructured N-terminal histone tail extending outwards from the nucleosome.⁴⁸ In addition to the core histones with histone tails, there are also linker histones that are less conserved and bind to the nucleosome with ~10bp of DNA at both the entry and exit sites of the nucleosome core particle.⁴⁶ The complex containing the nucleosome and a linker histone is called the chromatosome core particle with ~167 bp DNA.⁴⁶ They strongly associate with the linker DNA, the segment of DNA between adjacent nucleosomes. Histone H1 is the predominant linker histone in fungi and is important to stabilize the nucleosome structure and interact with neighboring nucleosomes by binding to the linker DNA.⁴⁹ In the nucleosome

and chromosome core particles, core histones - especially their histone tails - and linker histones primarily depend on the positively charged arginine and lysine residues to form electrostatic interactions with the backbone phosphates of DNA.⁵⁰ They play a crucial role in the interconversion between heterochromatin and euchromatin and serve as primary regulatory targets for chromatin-mediated control of secondary metabolism.

The epigenetic regulation of secondary metabolism through chromatin modification mainly encompasses the methylation of cytosines in DNA and the post-translational modifications (PTMs) of the histone tails and linker histone proteins (Figure 3).²⁰⁻²² These modifications play crucial roles in modulating chromatin structure and function, thereby influencing gene expression and metabolic pathways. In particular, PTMs allow nuclear modifier enzymes to interact with amino acid residues in the histone tails, adding or removing chemical groups and even small molecular weight proteins, such as acetyl and methyl units, phosphate, ubiquitin, and ADP-ribose groups.⁵¹ These specific chemical modifications also refer to “histone mark”.⁵² Currently, DNA methylation and hypomethylation of the C-5 position of the cytosine nucleotide, predominately within CpG dinucleotides, are well recorded,⁵³ while the histone acetylation and deacetylation of lysine residues on H3 or H4 histone tails are the most thoroughly studied PTMs.⁵⁴ Furthermore, the addition or removal of methyl groups to conserved lysine and arginine residues on histones represents another significant class of PTMs that are commonly encountered.⁵¹ The locations and combinations of these epigenetic modifications on the genome intertwine and influence gene expression.⁵⁵

To denote a histone PTM succinctly, the histone name is listed first, followed by the single-letter code for the modified amino acid residue, its position from the N-terminus of the histone tail, and finally, the type of modification.²² For example, the acetylation of the sixteen lysine residues on histone H4 is written as H4K16ac. The complex combination of PTMs on

histones is called histone code,⁵⁶ which is crucial in regulating gene expression. Histone code is regulated by three main types of enzymes,²² including “writers” that add specific PTMs to histone tails, “erasers” that remove these PTMs, and “readers” that recognize specific PTMs and mediate the downstream responses, such as transcriptional activation or repression (for more information, refer to section 5 in this review). Molecular-based and chemical epigenetic methods targeting these well-established chromatin-modifying enzymes, especially “writers” and “erasers,” have shown great potential for rationally directing BGC activation or suppression in fungi.^{57,58}

4. Methods for probing chromatin modification and secondary metabolism in fungi

Standard techniques for detecting chromatin modifications in fungi include western blotting, liquid chromatography-mass spectrometry (LC-MS), and chromatin immunoprecipitation (ChIP), each offering distinct advantages in epigenetic analysis (Figure 4). Some excellent reviews have summarized the methodologies for detecting chromatin modifications by LC-MS/MS,⁵⁹⁻⁶¹ and ChIP.^{22,62} Western blotting is a primary method for identifying specific histone modifications through antigen-antibody recognition. By employing primary antibodies targeting specific PTMs (e.g., H3K56ac, H3K27me3, H3K4me1/me2/me3),⁶³⁻⁶⁵ followed by enzyme-conjugated or fluorophore-labeled secondary antibodies, this technique enables assessment of modification levels through densitometric analysis of chemiluminescent or fluorescent signals. It is relatively simple and intuitive, allowing for a quick assessment of whether a specific histone modification exists and its approximate level in a sample.⁶³⁻⁶⁵ In contrast, LC-MS/MS can provide high-resolution characterization of histone PTMs through bottom-up, middle-down, and top-down proteomics workflows.⁵⁹ Among them, the bottom-up MS strategy is widely adopted and exploited. Following histone extraction, isolation,

chemical derivatization, and enzymatic digestion, reversed-phase chromatography separates peptide mixtures before MS analysis.⁵⁴ It can identify site-specific identification of modifications in fungi, such as distinguishing H3K9me from H3K9ac.⁶⁶ Moreover, with the help of isotope labeling techniques, MS/MS could potentially help study the dynamic changes of fungal histone modifications.⁶⁷ In addition, LC-MS/MS can be used to detect global DNA modifications after a DNA hydrolysis procedure by which genomic DNA is converted into mononucleosides.⁶⁸

Although western blotting and LC-MS can provide information about the alterations in the global levels of chromatin modifications, they cannot disclose the histone PTMs at specific genomic loci.²² In contrast, chromatin Immunoprecipitation (ChIP) is a powerful technique for studying the interactions between proteins and DNA (Figure 4). It has been frequently employed in investigating the specific histone PTMs at particular loci in fungi.⁶⁹ This process begins with the reversible cross-linking of protein and DNA, stabilizing their interactions. Next, the chromatin is fragmented into smaller pieces, usually through sonication or enzymatic digestion. Specific antibodies targeting the proteins of interest (PTMs such as H3K9ac, H3K14ac, H4K16ac, and H3K27me) are further added to pull down DNA-protein complexes.^{64,70-72} Subsequently, the cross-links are reversed to separate the DNA from the protein, and the DNA is then purified. Finally, DNA quantification can be carried out for several genomic regions by multiple qPCRs^{70,72} or at a genome-wide scale by sequencing technology (ChIP-seq),^{64,71} determining the genomic regions where the protein binds.

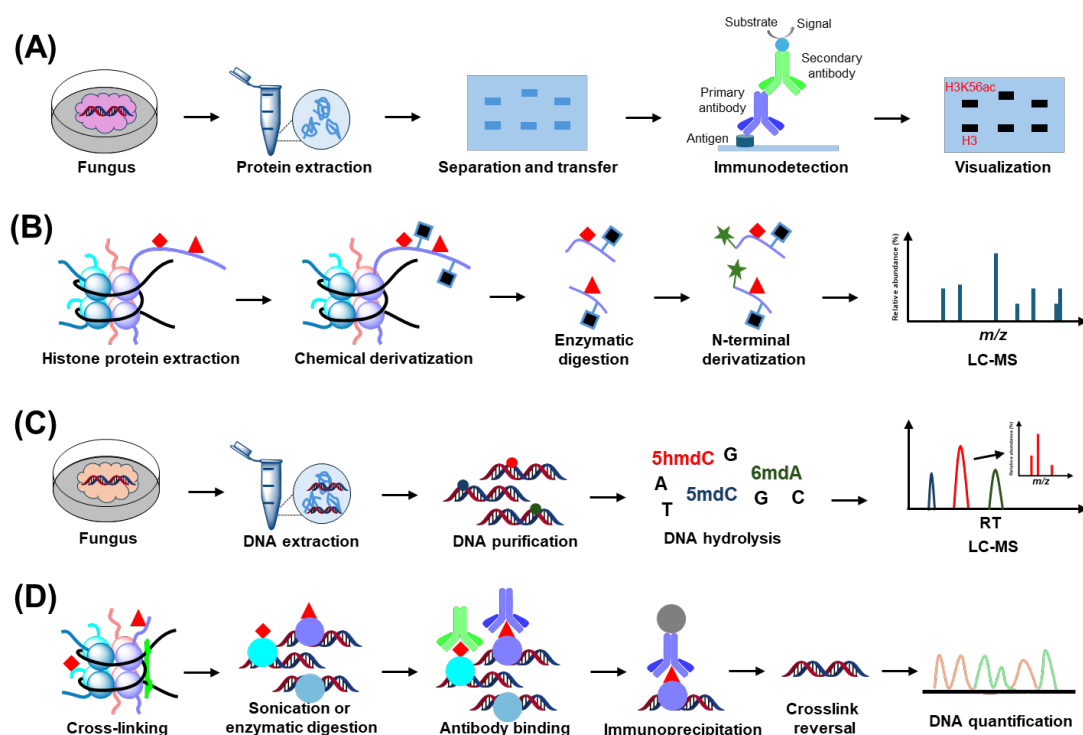


Figure 4. Methods for probing chromatin modifications in fungi. (A) Western blotting for identifying histone modifications through antigen-antibody recognition; (B) The bottom-up LC-MS strategy for identifying specific histone modifications; (C) LC-MS for detecting global DNA methylations after a DNA hydrolysis procedure by which genomic DNA is converted into mononucleosides; (D) Chromatin Immunoprecipitation (ChIP) for investigating the specific histone PTMs at particular loci.

In the context of chromatin modifications in fungi, many analytical techniques can be utilized to explore their secondary metabolism. The framework for analyzing and discovering secondary metabolites has evolved significantly. Rather than depending on the traditional bioactivity-guided approaches, research efforts have now shifted towards genomics,⁷³ metagenomics,⁷⁴ as well as MS- and NMR-based metabolomics,⁷³ together with their integrated applications.⁷⁵ For example, our research group has effectively employed LC-MS to uncover the production of decalin-containing compounds following the epigenetic modulation of the endophytic fungus *Eupenicillium* sp. LG41.⁷⁶ This innovative approach has led to the rapid identification of two promising new derivatives. Moreover, MS-based metabolic profiling of the endophytic fungus *Chaetomium nigricolor* F5 has proven instrumental in the

analysis and isolation of novel cytochalasans.⁷⁷ Utilizing these advanced analytical methods along with epigenetic stimulation presents significant opportunities to enhance the potential applications of fungal endophytes across various fields.

5. Mechanistic insights into epigenetic remodelling of silent gene clusters

The epigenetic mechanisms responsible for activating silenced biosynthetic gene clusters (BGCs) in fungi includes histone acetylation, histone methylation and DNA methylation. These key biomacromolecules provide the essential context for the targeted activation of dormant pathways in endophytic fungi.

5.1. Histone acetylation

Histone acetylation is the most studied PTM in fungi for epigenetic remodeling of secondary metabolism and is commonly associated with actively transcribed chromatin.⁷⁸ There are two primary types of enzymes involved in the acetylation and deacetylation of lysine within the epigenome, namely histone acetyltransferases (HATs, “writers”) and histone deacetylases (HDACs, “erasers”).⁷⁹ HATs facilitate the transfer of an acetyl group from acetyl-CoA to a lysine residue on the histone tails (Figure 3). This process activates a BGC through histone acetylation by neutralizing the positive charge of lysine, which generally reduces the electrostatic interactions between the histone and the negatively charged DNA.⁵⁰ Such modifications that decrease the interaction of lysines with DNA are crucial for the transition between ‘open’ euchromatin, which promotes gene expression, and ‘closed’ heterochromatin, which is associated with gene silencing.⁵⁰ In contrast, HDACs remove acetyl groups from lysine residues, forming more condensed heterochromatin, which can result in transcriptional repression by making the DNA less accessible to the transcription machinery.⁷⁹ HDACs have been grouped into four classes: Class I (HDACs 1-3 and 8), Class II (HDACs 4-7, 9, and 10), Class III, and Class IV (HDAC 11).^{80,81} HDAC Class III, as the sirtuins family, are nicotinamide adenine

dinucleotide (NAD⁺) dependent deacetylases, whereas the other three ones remove acetyl groups in a zinc-dependent manner using a divalent zinc cation for catalysis. It has to be noted that HDAC11, as the only member of Class IV with a very efficient long-chain fatty acid deacetylase activity, has not been identified in fungi and are present in humans and plants.⁸²

5.2 Histone methylation

Histone methylation in fungi is a well-characterized post-translational modification (PTM) that plays a critical role in regulating transcriptional outcomes, mediating both activation and repression in a complex and often nuanced manner.⁸³ This modification is primarily executed by histone methyltransferases (HMTs), commonly referred to as “writers,” which include histone lysine methyltransferases (HKMTs) and protein arginine methyltransferases (PRMTs) (Figure 3).⁸³ HKMTs can be subdivided into two distinct classes: SET domain-containing methyltransferases, such as SUV39 and SET1, which utilize their SET domain as the catalytic site and are associated with transcriptional activation of diverse genes, and the DOT1 family, which methylates K79 on histone H3 with a role in cell development, DNA damage repair and metabolic process.⁸⁴ These enzymes facilitate the transfer of one to three methyl groups from the methyl donor S-adenosyl-methionine (SAM) to lysine residues, resulting in mono-, di-, or tri-methylated forms.^{83,84} Within the PRMT family, two major subclasses are acknowledged: type I and type II PRMTs. Type I PRMTs are responsible for adding single methyl groups to arginine residues and generating asymmetric N,N'-dimethylarginine, while type II PRMTs catalyze the formation of symmetric N,N-dimethylarginine.²² The reverse process—demethylation—is carried out by histone demethylases (HDMTs), or “erasers.” These enzymes are classified into two principal subgroups: lysine-specific demethylases (like LSD1), which perform oxidative demethylation of lysine residues, and Jumonji C domain-containing

demethylases (JMJC), adept at removing all three methyl groups from specific lysines.²²

5.3 DNA methylation

DNA methylation, a prevalent post-translational modification (PTM) in fungi, is predominantly linked to gene silencing and can be categorized into three types: C5-methylcytosine (5mC),⁸⁵ N4-methylcytosine (4mC),⁸⁵ and N6-methyladenine (6mA) (Figure 3).⁶⁸ Among these, 5mC is the most extensively studied form of methylation, with its dysregulation frequently implicated in various human diseases, particularly oncogenesis.⁸⁶ The regulatory enzymes, DNA methyltransferases (DNMTs), known as “writers,” orchestrate the methylation of bases, generating the corresponding methylated derivatives of cytosines or adenines.

DNMTs can be categorized into five classes, namely DNMT1 (which includes DNMT1/Masc2, MET1, CMT, and Dim-2), DNMT2, DNMT3 (including DNMT3 and DRM), Masc1/RID, and DNMT5.⁸⁷ Notably, DNMT3 and Masc1/RID are key players in “*de novo*” methylation, targeting initially unmethylated cytosines to establish novel DNA methylation landscapes.⁸⁷ Conversely, DNMT1 and DNMT5 are primarily involved in “maintenance” methylation, preserving the established methylation patterns through DNA replication. Additionally, DNMT2 is known to methylate transfer RNA specific for aspartic acid (tRNA^{Asp}) across all domains of life.⁸⁸ It is important to note that DNMT3 is absent in fungal species, while Masc1/RID is fungi-specific. The predominant contexts for methylation in fungi encompass CG, CHG, and CHH, particularly within transposon regions. The presence of various DNMT types across different fungal genomes results in substantial variability in methylation patterns, which are associated with a range of complex biological processes.⁸⁷

The pathways for DNA demethylation display significant variability across mammals and plants, while they remain poorly characterized in fungi.

Unlike the relatively straightforward process of DNA methylation, which typically involves the addition of methyl groups to cytosines or adenines, the catalytic removal of these methyl units is notably more complex in eukaryotes. A pertinent example is CcTet, a bifunctional ten-eleven translocation (Tet) dioxygenase derived from the mushroom *Coprinopsis cinerea* (Psathyrellaceae), which engages in both 5mC and 6mA demethylation via distinct mechanisms (Figure 3).⁸⁹ Specifically, CcTet oxidizes the methyl group of 5mC to generate 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC).⁹⁰ The oxidation products 5fC and 5caC are likely substrates for recognition and excision by thymine DNA glycosylase (TDG), with subsequent restoration to unmethylated cytosine via the base excision repair (BER) pathway, as observed in mammalian systems.⁸⁹ Furthermore, CcTet catalyzes the oxidation of 6mA to the intermediate 6-hydroxymethyladenosine (6hmA), thereby facilitating the subsequent demethylation process.⁹¹

6. Design and synthesis of small-molecule chemical epigenetic modulators

Epigenetic modifications play a significant role in regulating metabolic pathways across various fungal species and are essential in the initiation and progression of multiple diseases, including cancer. Research has increased in developing small-molecule inhibitors that specifically target epigenetic regulators.⁹²⁻⁹⁴ These chemical entities may modulate epigenetic marks within endophytic fungi, providing insights into their metabolic pathways.

6.1 “Cap-Linker-ZBG” type HDAC inhibitors

When Marks and Breslow cultured murine erythroleukemia cells (MELC) for transfection with 280 mM dimethyl sulfoxide (DMSO) in aqueous buffer, around two-thirds of the cells turned red, revealing the hemoglobin formation.⁹⁵

In efforts to unravel the underlying mechanism by which DMSO induces erythroid differentiation in MELC, researchers identified suberoylanilide hydroxamic acid (SAHA), widely known as vorinostat, a prominent histone deacetylase (HDAC) inhibitor and an FDA-approved anticancer agent. SAHA's structural composition features a phenyl ring-containing Cap that interacts with the surface of the HDAC enzyme, promoting robust binding within its hydrophobic pocket.⁹⁴ The Zinc-Binding Group (ZBG), consisting of a hydroxamic acid moiety, chelates the zinc ion present at the catalytic site of the HDAC, thereby directly inhibiting its enzymatic activity. A six-carbon aliphatic Linker connects the Cap and ZBG, optimizing the spatial configuration to facilitate the inhibitor's access to the HDAC catalytic tunnel.⁹⁵ The synthetic pathway for SAHA involves the condensation of suberic anhydride with anilide hydroxamic acid, with the introduction of the phenyl substituent to enhance its inhibitory potency.^{94,95} SAHA effectively targets class I and II HDACs, resulting in the acetylation of histones and non-histone proteins, thus regulating gene expression and triggering apoptotic pathways in cancer cells. Both preclinical and clinical investigations have demonstrated its efficacy across various malignancies, culminating in FDA approval in 2006.⁹⁵ Subsequently, an increasing array of HDAC inhibitors have been designed following the "Cap-Linker-ZBG" template established by SAHA (Figure 5).

6.1.1 Cap-based design and synthesis

The design and modification of "cap" units have been extensively documented, highlighting their significant potential for enhancing selectivity and epigenetic modulation. Belinostat (**1**, Figure 5), an FDA-approved HDAC inhibitor featuring a sulfonamide-hydroxamide "cap" motif, exhibits improved enzymatic inhibition attributed to its distinctive chemical architecture.⁹⁶ The cap modification, specifically defined by the phenylsulfamoyl group, enhances target affinity and selectivity by promoting interactions with the active sites of

HDAC enzymes. This structural characteristic is instrumental in its preferential cytotoxicity against malignant cells relative to normal cell populations, consistent with findings from preclinical investigations.⁹⁶ Notably, alterations in the phenyl ring substituents to incorporate triazole phenyl groups have shown a marked influence on selectivity for HDAC1 and HDAC6.⁹⁷ Among the derivatives evaluated, compound **2** (Figure 5), featuring a triazole phenyl cap, exhibited the highest selectivity for HDAC6, achieving a remarkable 51-fold preference.

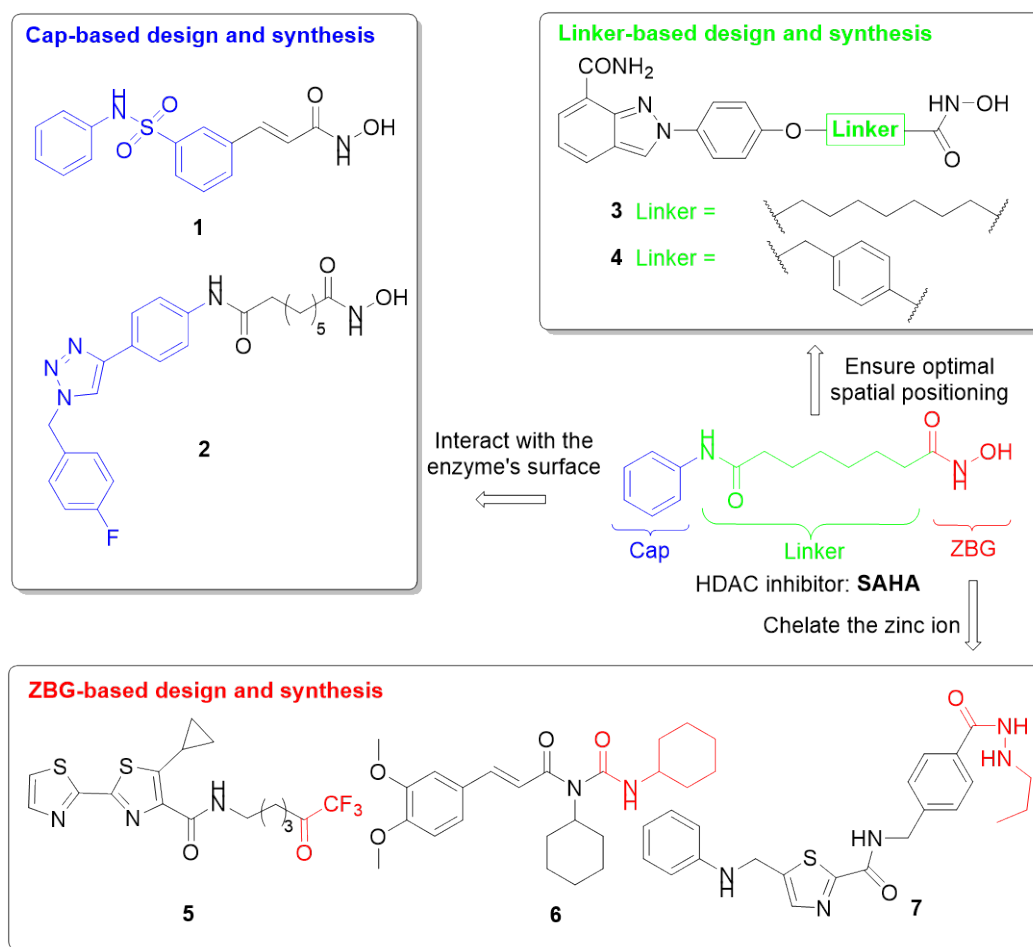


Figure 5. The design and synthesis of “Cap-Linker-ZBG” type HDAC inhibitors (**1-7**) based on the FDA-approved first HDAC inhibitor, SAHA.

6.1.2 Linker-based design and synthesis

The design of HDAC inhibitors prominently incorporates the “linker” component. In their study, Chi et al. concentrated on developing and synthesizing PARP-1/HDAC dual-targeting inhibitors through a linker-based approach, leveraging benzopyrazole and benzimidazole scaffolds.⁹⁸ A total of fourteen compounds were synthesized, incorporating a variety of linkers such as benzene and alkyl chains to refine target interactions. Notably, compounds **3** and **4** (Figure 5), which featured benzene-containing linkers, exhibited enhanced antiproliferative activity and improved membrane permeability relative to those with alkyl linkers. This improvement can be attributed to their superior binding affinity and lower polarity.⁹⁸

6.1.3 ZBG-based design and synthesis

Enhancing the deacetylation-inhibitory efficacy can be achieved through modifications to the ZBG. Gong et al. developed bisthiazole-based HDAC inhibitors by systematically varying the ZBG and the linking chains (Figure 5).⁹⁹ The synthetic pathway encompassed several key steps: esterification, reduction, oxidation, the Wittig reaction, methylsilylation, hydrolysis, and condensation. Notably, compound **5** (Figure 5), featuring a trifluoromethyl ketone as the ZBG, exhibited significant antiproliferative effects against various cancer cell lines.⁹⁹ Additionally, derivatives of ferulic acid, synthesized through esterification, methylation, and hydrolysis, were assessed using the Glide module in Schrödinger software.¹⁰⁰ Among these, compound **6** (Figure 5)—with an amide *n*-hexane ZBG—emerged as a promising HDAC inhibitor, showing potential to suppress lung cancer A549 cell proliferation and offering a novel candidate for cancer treatment.¹⁰⁰ Furthermore, HG-30 (**7**, Figure 5), a hydrazide-based HDAC3 inhibitor, utilized ZBG modification to achieve selective inhibition through chelation of the catalytic Zn²⁺ ion.¹⁰¹ The hydrazide ZBG not only enhances the pharmacokinetic profile, including a favorable

bioavailability ($F = 57\%$) and a benign toxicity profile ($LD_{50} > 1000$ mg/kg), but also improves solubility and metabolic stability.

6.2 Specific chemical scaffold-based epigenetic modifiers

Epigenetic modifiers with unique structural skeletons, unrelated to the Cap-Linker-ZBG framework, also warrant our attention. Romidepsin (**8**, Figure 6), a naturally derived and selective inhibitor of class I and II HDAC, received FDA approval in 2009 for the treatment of cutaneous T-cell lymphoma (Figure 6).¹⁰² Originally isolated from *Chromobacterium violaceum*, the synthetic pathway for compound **8** involves the construction of a hydroxy-mercapto heptenoic acid moiety and culminates in the formation of a 16-membered cyclic depsipeptide.¹⁰² Tripartin (**9**, Figure 6), another natural product, functions as a HDMT inhibitor by elevating levels of H3K9me3 and H3K9me2.¹⁰³ Its unique structural feature is a dichloromethylcarbinol group connected to a dihydroxyindandione scaffold, setting it apart from conventional small-molecule inhibitors. The synthesis of compound **9** entails some crucial steps, including the addition of a dichloromethyl anion to dimethoxyindanone under cryogenic conditions, followed by benzylic oxidation using $Pd(OAc)_2/TBHP$, and finally, $AlCl_3$ mediated demethylation.¹⁰³ Compound **10** (Figure 6), which incorporates an $\alpha \rightarrow \beta$ -amino acid substitution leading to a 13-membered ring, serves as a versatile framework for developing highly potent and selective HDAC inhibitors.¹⁰⁴ Its synthesis employs solid-phase combinatorial library techniques using “one-bead–one-compound” strategies. Strategic structural modifications, such as the introduction of aromatic residues and optimization of Zn^{2+} -binding groups, have resulted in the generation of isoform-selective inhibitors.¹⁰⁴

6.3 DNA-DBDs type for enhancing affinity

In contrast to monomeric epigenetic regulators, sequence-specific epigenetic regulators represent advanced synthetic dual-target ligands with the ability to modulate multi-gene networks. DNA-binding domains (DBDs), particularly pyrrole-imidazole polyamides (PIPs), exhibit a strong affinity for specific DNA sequences (Figure 6).¹⁰⁵ The development of DBD-based inhibitors and activators of HATs and HDACs through conjugation with epigenetic modulators has emerged as a robust research area.¹⁰⁶ Yu et al. designed DBD-HAT inhibitors, referred to as C646-PIPs, which featured varied linker lengths and were synthesized using solid-phase methodologies.¹⁰⁶ Notably, compound **11** (Figure 6) demonstrated a high degree of affinity and specificity for its DNA target, effectively inhibiting HAT activity while displaying antiproliferative effects in p53 wild-type cancer cell lines.

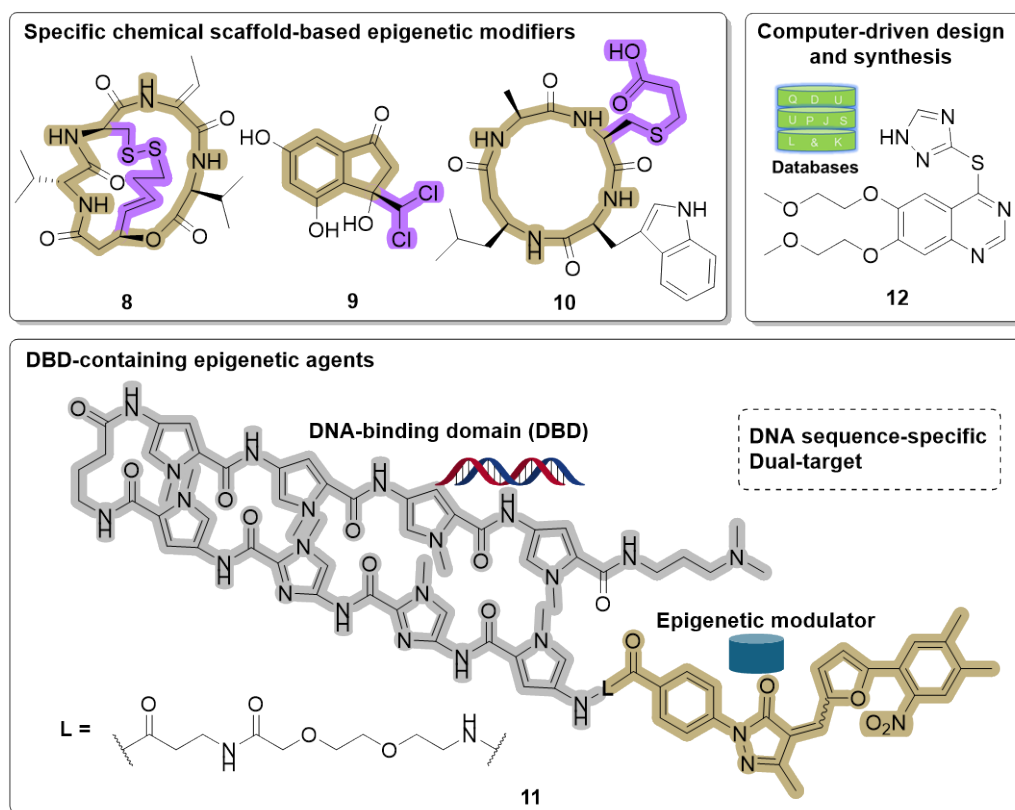


Figure 6. The design and synthesis of small molecule chemical epigenetic modulators (**8-12**) based on specific chemical scaffolds, DBD, and large databases.

6.4 Computer-driven analysis and design

The HEMD database catalogs a comprehensive set of 269 epigenetic enzymes, derived from PubMed abstracts combined with manual curation, offering detailed annotations encompassing genetic data, biological functions, associated mutations, and related pathologies.¹⁰⁷ In addition, the database includes an extensive list of 4,377 chemical modulators, complete with information on binding sites, pharmacological profiles, and therapeutic applications, thereby facilitating advancements in epigenetic therapeutic research.¹⁰⁷ A recent study by Li et al. employed a drug repurposing approach to discover a novel series of potent lysine-specific demethylase 1 (LSD1) inhibitors, specifically quinazoline derivatives.¹⁰⁸ By screening an existing compound library, they identified several LSD1 inhibitors and subsequently developed compound **12** (Figure 6) through targeted chemical modifications. This compound demonstrated excellent aqueous solubility and markedly enhanced inhibitory efficacy against LSD1 in both *in vitro* and *in vivo* assays.¹⁰⁸ Additionally, the study reported significant effects on tumor cell dynamics, including reduced proliferation, migration, and induction. A computer-assisted approach was utilized to construct a predictive AI model for identifying compounds that selectively target HDAC1 activity.¹⁰⁹ The methodology involved creating a pharmacophore model via Phase software, conducting rigorous conformational analysis using MacroModel, and generating a robust 3D-QSAR model based on a dataset of 370 HDAC inhibitors.

7. Chemical modulation of endophytic fungal epigenetic machinery

Fungal epigenetic mechanisms encompass intricate and dynamic regulatory transcriptional pathways governed by the covalent modifications of chromatin, mediated by enzymes classified as writers, erasers, and readers.²² Various naturally occurring and synthetic small molecules, termed epigenetic modulators or elicitors, can inhibit these DNA methylation and histone post-translational modification (PTM) enzymes.¹¹⁰ This domain of research is

collectively known as chemical epigenetics.¹¹¹ Recent developments in chemical epigenetics highlight the potential to exploit the metabolic functionalities of fungal endophytes.¹¹¹ Small chemical elicitors, such as the frequently used HDAC inhibitor, SAHA, and the DNMT inhibitor, 5-azacytidine (Figure 7), can trigger epigenetic alterations in these endophytic fungi, activating dormant biosynthetic gene clusters (BGCs).^{112,113} Such activation can enhance the production of known metabolites or facilitate the biosynthesis of novel compounds (Tables 1 and 2). Since the initial application of chemical epigenetics in fungi in 2008,¹¹⁴ followed by its first extension to fungal endophytes in 2012,¹¹³ chemical epigenetics holds significant promise for advancing research across agriculture, medicine, and environmental sustainability.

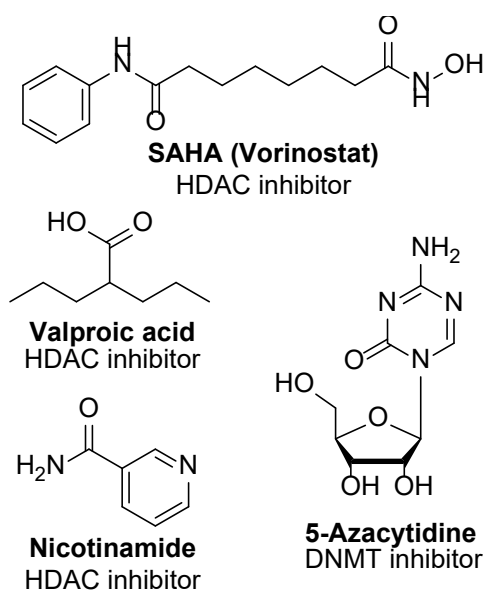


Figure 7. Epigenetic modulators used for the activation of silent or low-expression BGCs in endophytic fungi.

7.1 HDAC inhibitors

Administering HDAC inhibitors to endophytic fungi presents a promising strategy for activating BGCs that are typically dormant or minimally expressed under standard laboratory conditions (Table 1). In their research, Chen et al. introduced the inhibitor SAHA into the culture medium of the endophytic fungus

Fusarium oxysporum f. sp. harbored by the medicinal plant *Datura stramonium* L.¹¹⁵ This treatment facilitated the isolation and identification of two novel fusaric acid derivatives (**13** and **14**, Figure 8), which were undetected in the absence of SAHA.¹¹⁵ Although these two compounds exhibited no antibacterial activity, they may represent oxidized derivatives of the toxic fusaric acid, suggesting that their biosynthesis could serve to mitigate the plant's toxicity.

Three HDAC inhibitors—SAHA, sodium butyrate, and valproic acid—were employed to modify the secondary metabolic profile of *Aspergillus fumigatus* (GA-L7), an endophytic fungus isolated from *Grewia asiatica* L.¹¹⁶ Notably, valproic acid significantly up-regulated the expression of several genes involved in the biosynthesis of fumiquinazoline C (**15**, Figure 8), leading to an approximately tenfold increase in its overall production.¹¹⁶ In a separate study, Gubiani et al. screened a small collection of fungal endophytes by culturing them in liquid media supplemented with or without the epigenetic modifier SAHA.¹¹⁷ The endophytic *Phoma* sp. nov. LG0217 was found to biosynthesize (10'S)-verruculide B (**16**, Figure 8), vermistatin (**17**), and dihydrovermistatin (**18**) specifically at a SAHA concentration of 500 μ M, whereas these secondary metabolites were not detected at lower concentrations of 100 and 200 μ M.¹¹⁷ Furthermore, compound **16** demonstrated inhibitory activity against protein tyrosine phosphatases, with IC₅₀ values ranging from 8.8 to 16.6 μ M.

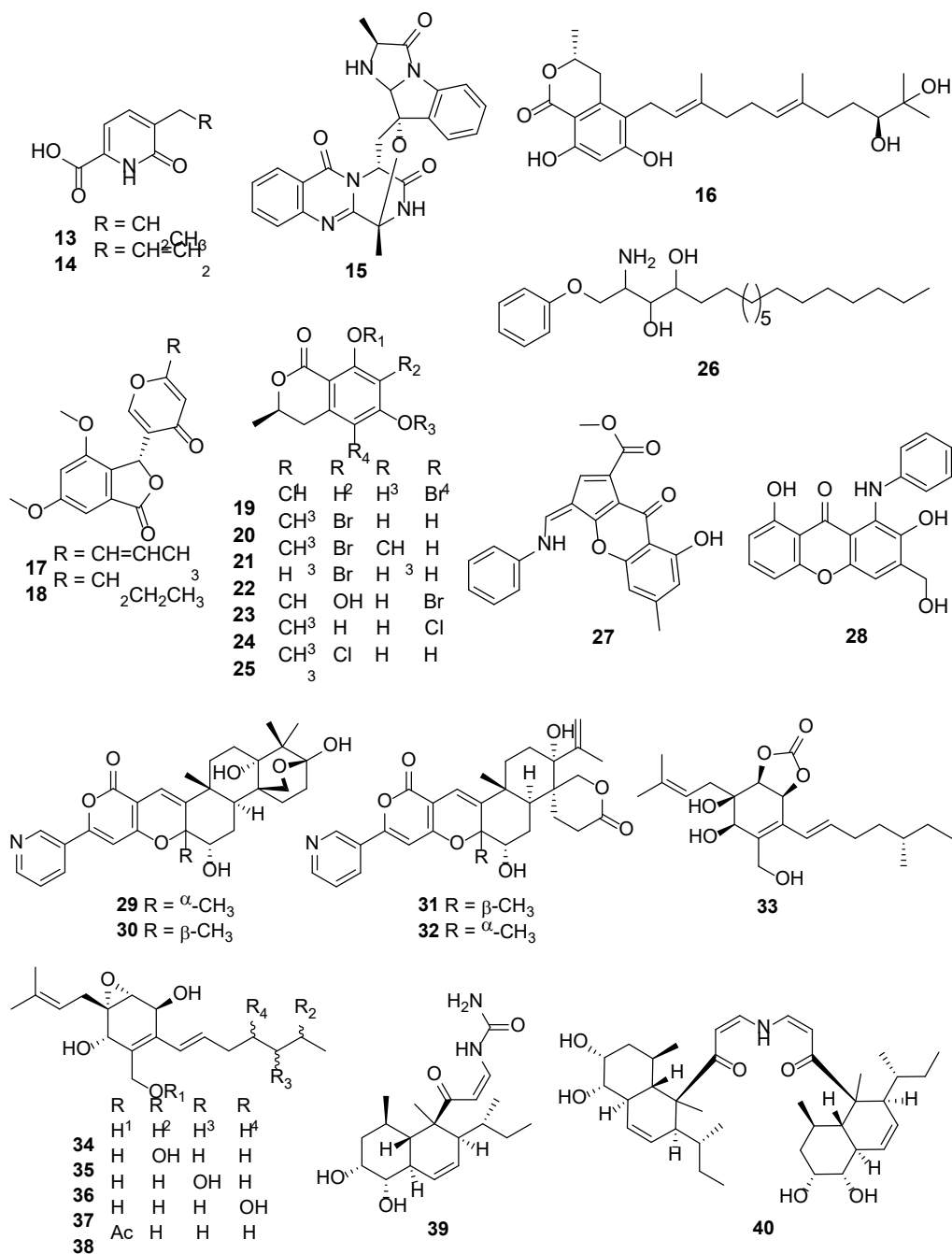


Figure 8. Chemical structures of compounds **13-40** induced by HDAC inhibitors in endophytic fungi.

Following a thorough pre-screening of various chemical epigenetic modifiers on endophytic fungi, Zhao et al. identified the endophytic fungus *Lachnum palmae*, isolated from *Przewalskia tangutica*, which exhibited sensitivity to SAHA.¹¹⁸ Further chemical analysis of this fungal strain in the

presence of SAHA led to the isolation of seven novel halogenated dihydroisocoumarins, designated as palmaerones A-G (**19-25**, [Figure 8](#)), which demonstrated antimicrobial, anti-inflammatory, and cytotoxic properties.¹¹⁸ In another study, the roots-derived endophytic fungus *Drechslera* sp., when treated with SAHA, triggered the release of hexosylphytosphingosine (**26**, [Figure 8](#)), a compound that was virtually undetectable in the control medium lacking SAHA.¹¹⁹ Moreover, the endophytic fungus *Penicillium chrysogenum* P03MB2, sourced from the medicinal plant *Albizia adianthifolia*, exhibited activation of silent biosynthetic gene clusters (BGCs) encoding cryptic natural products with anti-HIV potential upon treatment with sodium butyrate.¹²⁰ Adpressa et al. also reported the discovery of the salt-tolerant endophytic fungus *Chalara* sp. 6661 from *Artemisia vulgaris*, which metabolizes SAHA to produce aniline.¹²¹ This aniline moiety can be further integrated into the fungal biosynthetic pathways to yield aniline-containing “unnatural” polyketides, specifically chalanilines A and B (**27** and **28**, [Figure 8](#)). The cultivation of an endophytic fungus *Penicillium* sp. KMU18029 with SAHA yielded two pairs of diterpenic meroterpenoids with an uncommon framework ([Figure 8](#)), pyrandecarurins A (**29**) and B (**30**), and pileotins A (**31**) and B (**32**).¹²² Similarly, six new polyketides were isolated from a mangrove-associated endophytic fungus *Diaporthe goulteri* by adding SAHA for the chemical epigenetic modification.¹¹² These polyketides are cytosporin E2 (**33**), cytosporin D1 (**34**), cytosporin G1 (**35**), cytosporin J1 (**36**), cytosporin K1 (**37**), and cytosporin F1 (**38**) ([Figure 8](#)).

Our research group utilized a nicotinamide-based HDAC inhibitor, specifically the NDA⁺-dependent variant, to investigate the impact of epigenetic modulation on the metabolic profiles of selected endophytic fungi.⁷⁶ Supplementation of the PDB medium with nicotinamide significantly enhanced the production of structurally intricate secondary metabolites, namely eupenicinols C and D (**39** and **40**, [Figure 8](#)).⁷⁶ This strategy proved

advantageous in the structural elucidation of compounds generally produced in minimal quantities under standard laboratory conditions.

7.2 DNMT inhibitors

DNMT inhibitors target and inhibit DNMTs, the key enzymes responsible for methylating cytosine and adenine residues in the DNA sequence.⁸⁵ By obstructing the activity of these enzymes, DNMT inhibitors effectively halt the methylation process, which facilitates the demethylation of previously methylated genomic regions. This demethylation can lead to the reactivation of silenced genes due to hypermethylation, potentially restoring their normal expression levels.⁵³ This mechanism underlies the therapeutic efficacy of DNMT inhibitors in various conditions, particularly in cancer, where they may reverse aberrant gene silencing and contribute to tumor suppression.⁸⁶

The DNMT inhibitor 5-azacytidine, at a concentration of 500 μ M, was introduced into the culture medium of the fungal endophyte *Pestalotiopsis crassiuscula*, isolated from the leaves of *Fragaria chiloensis* (Table 2).¹²³ This treatment significantly modulated the secondary metabolite profile of the fungus, leading to the isolation and identification of three compounds (Figure 9): 4,6-dihydroxy-7-hydroxymethyl-3-methoxymethylcoumarin (**41**), pestalotiopyrone G (**42**), and scirpyrone B (**43**).¹²³ Among them, compound **41** was a new compound, while the production of compounds **42** and **43** was enhanced. In addition, 5-azacytidine was utilized to epigenetically remodel the fungal genome and activate cryptic biosynthetic pathways in the endophytic fungus *Chaetomium globosporum*, which resides in *Euphorbia humifusa*.¹²⁴ This approach facilitated the discovery of two new cyclopentenones, globosporins A and B (**44** and **45**, Figure 9), alongside two new monoterpene indole alkaloids, globosporines C and D (**46** and **47**, Figure 9). Notably, compound **47** exhibited significant activity as a potent antiphytopathogenic agent.

Table 1. HDAC inhibitors for modulating endophytic fungal epigenetic machinery.

Modifiers	Cons.	Endophytic fungi	Host plants	Induced or enhanced compounds	Biological activity	Ref.
SAHA	500 μ M	<i>Fusarium oxysporum</i> f. sp.	<i>Datura stramonium</i> L.	5-Butyl-6-oxo-1,6-dihydropyridine-2-carboxylic acid (13), 5-(but-9-enyl)-6-oxo-1,6-dihydropyridine-2-carboxylic acid (14)	Antibacterial	115
SAHA	500 μ M	<i>Phoma</i> sp. nov. LG0217	<i>Parkinsonia microphylla</i>	(10'S)-Verruculide B (16), vermistatin (17), dihydrovermistatin (18)	Protein tyrosine phosphatases inhibition	117
SAHA	500 μ M	<i>Lachnum palmae</i>	<i>Przewalskia tangutica</i>	Palmaerones A-G (19-25)	Antimicrobial, anti-inflammatory, cytotoxicity	118
SAHA	500 μ M	<i>Drechslera</i> sp.	<i>Morus Cathayana Hemsl.</i>	Hexosylphytosphingosine (26)	Antifungal	119
SAHA	1 mM	<i>Chalara</i> sp. 6661	<i>Artemisia vulgaris</i>	Chalanilines A and B (27 and 28)	Antibacterial	121
SAHA	100 μ M	<i>Penicillium</i> sp. KMU18029	<i>Aconitum brachypodum</i> Diels.	Pyrandecarurins A and B (29 and 30), pileotins A and B (31 and 32)	Acetylcholinesterase inhibition	122
SAHA	1 mM	<i>Diaporthe goulteri</i>	<i>Acanthus ilicifolius</i> L.	Cytosporins E2 (33), D1 (34), G1 (35), J1 (36), K1 (37), F1 (38)	Antioxidant	112
Valproic acid	500 μ M	<i>Aspergillus fumigatus</i>	<i>Grewia asiatica</i> L.	Fumiquinazoline C (15)	None reported	116
Nicotinamide	1.2 mM	<i>Eupenicillium</i> sp. LG41	<i>Xanthium sibiricum</i>	Eupenicinols C and D (39 and 40)	Antibacterial, cytotoxicity	76

A novel antibacterial polyketide, identified as (3*S*,4*R*)-3,4,8-trihydroxy-6-methyl-3,4-dihydronaphthalen-1(2*H*)-one (**48**, Figure 9), was isolated from the endophytic fungus *Penicillium* sp. KMU18029 following treatment with the DNMT inhibitor 5-azacytidine.¹²⁵ Utilizing ¹H NMR-based metabolomics, Happyana et al. demonstrated that procaine, another DNMT inhibitor, significantly altered the metabolite profiles of the endophytic fungus *Talaromyces wortmannii* isolated from *Morus cathayana* Hemsl.¹²⁶ Notably, procaine induced the biosynthesis of mannitol, choline, lactic acid, and phosphocholine, while simultaneously repressing the production of various fatty acids, including palmitic, stearic, linoleic, and oleic acids.¹²⁶

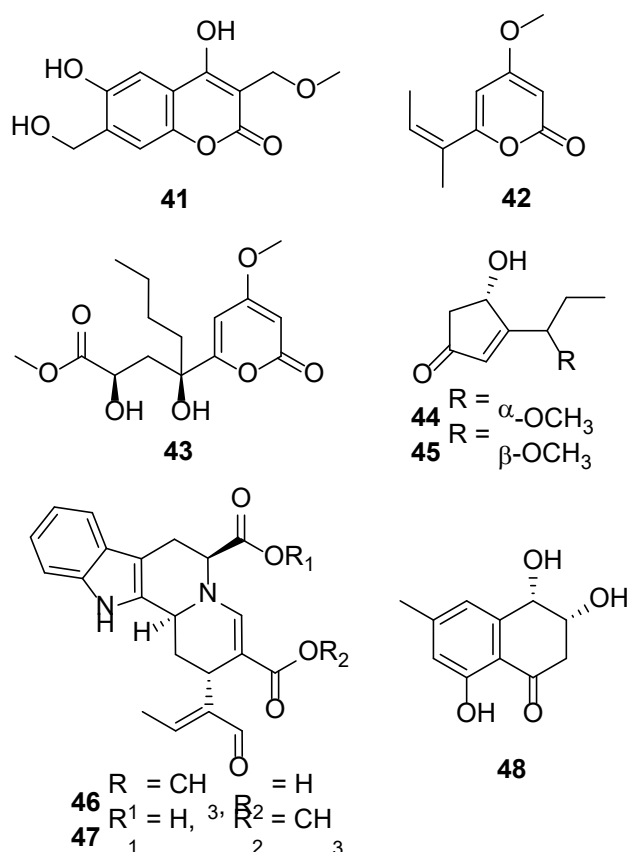


Figure 9. Chemical structures of compounds **41-48** induced by DNMT inhibitors in endophytic fungi.

7.3 The combination of HDAC and DNMT inhibitors

Intriguingly, a synergistic effect was observed when HDAC and DNMT inhibitors were combined, resulting in enhanced production of specific fungal

metabolites compared to using either inhibitor in isolation (Table 2).^{113,127} The Abe group reported that applying 5-azacytidine and SAHA to the endophytic fungus *Alternaria* sp. led to significant alterations in its metabolic profile, notably inducing the production of mycotoxins. The yield of (5*S*,8*S*)-tenuazonic acid (**49**, Figure 10), which was absent under standard cultivation conditions, was notably doubled when compared to cultures supplemented solely with SAHA.¹¹³ Furthermore, employing the same epigenetic modifiers to activate cryptic BGCs in another plant-associated endophytic fungus, *Pestalotiopsis acacia*, enabled the isolation of three novel aromatic compounds (Figure 10): 2'-hydroxy-6'-hydroxymethyl-4'-methylphenyl-2,6-dihydroxy-3-(2-isopentenyl)benzoate (**50**), 4,6-dihydroxy-7-hydroxymethyl-3-methylcoumarin (**51**), and 4,6-dihydroxy-3,7-dimethylcoumarin (**52**).¹²⁷

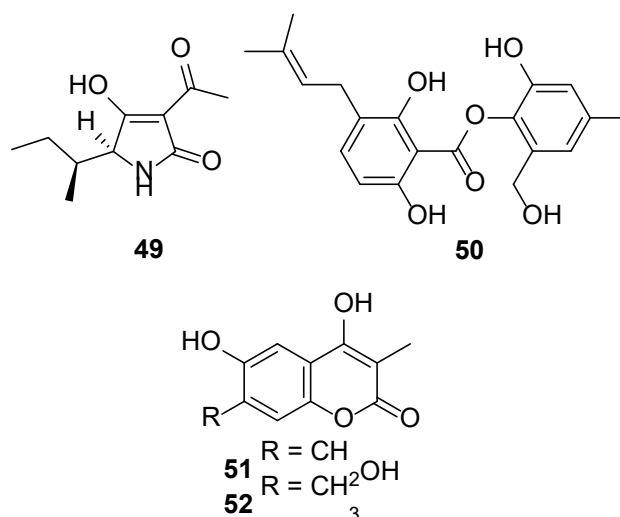


Figure 10. Chemical structures of compounds **49-52** induced by the combined use of HDAC and DNMT inhibitors in endophytic fungi.

Table 2. DNMT inhibitors used alone or in combination with HDAC inhibitors for modulating endophytic fungal epigenetic machinery.

Modifiers	Cons.	Endophytic fungi	Host plants	Induced or enhanced compounds	Biological activity	Ref.
5-azacytidine	500 µM	<i>Pestalotiopsis crassiuscula</i>	<i>Fragaria chiloensis</i>	4,6-Dihydroxy-7-hydroxymethyl-3-methoxymethylcoumarin (41), pestalotiopyrone G (42), scirpyrone B (43)	Antifungal	123
5-azacytidine	500 µM	<i>Chaetomium globosporum</i>	<i>Euphorbia humifusa</i>	Globosporins A and B (44 and 45), globosporines C and D (46 and 47)	Antimicrobial	124
5-azacytidine	100 µM	<i>Penicillium</i> sp. KMU18029	<i>Aconitum brachypodum</i> Diels.	(3 <i>S</i> ,4 <i>R</i>)-3,4,8-Trihydroxy-6-methyl-3,4-dihydronaphthalen-1(2 <i>H</i>)-one (48)	Antibacterial	125
5-azacytidine and SAHA	250 µM and 500 µM	<i>Alternaria</i> sp.	<i>Datura stramonium</i> L.	Tenuazonic acid (49)	None reported	113
5-azacytidine and SAHA	500 µM and 500 µM	<i>Pestalotiopsis acaciae</i>	unknown	2'-Hydroxy-6'-hydroxymethyl-4'-methylphenyl-2,6-dihydroxy-3-(2-isopentenyl)benzoate (50), 4,6-dihydroxy-7-hydroxymethyl-3-methylcoumarin (51), 4,6-dihydroxy-3,7-dimethylcoumarin (52)	None reported	127

8. Inactivation or overexpression of epigenetic regulators

Molecular epigenetic modification enables precise manipulation of the epigenetic landscape, including the targeted deletion or overexpression of chromatin-modifying enzymes in endophytic fungi. Chemical epigenetic modifications have demonstrated efficacy in reactivating dormant BGCs within endophytic fungi, highlighting their potential for natural product discovery. Nevertheless, these modifications often fail to fully activate cryptic gene clusters, leading to variability and unpredictability in the outcomes.⁵⁸ A review by Pfannenstiel and Keller has underscored the role of chromatin remodeling proteins in regulating secondary metabolism in fungi, presenting compelling targets for genetic modification.²² For example, the inactivation of HDACs has emerged as an effective strategy, facilitating a level of specificity that traditional methods may not achieve.²⁵ It is crucial to acknowledge that the interplay of various epigenetic modifications can either enhance or suppress gene expression, thus contributing to a complex regulatory framework.²⁵

8.1 Erasers (HDACs)

Shwab et al. first reported the disruption of epigenetic regulators in 2007.¹²⁸ The class II HDAC HdaA is a pivotal epigenetic modification enzyme in the molecular modulation of secondary metabolite production. Its regulation exhibits a dual role, exerting both positive and negative influences. For instance, in *Aspergillus nidulans*, HdaA has been shown to downregulate the expression of sterigmatocystin and penicillin,¹²⁸ while it also diminishes the transcription of nonribosomal peptide synthetase (NRPS) genes in *Aspergillus fumigatus*.¹²⁹ Conversely, the overexpression of *hdaA* leads to increased gliotoxin production, a notable virulence factor, whereas the *hdaA* gene disruption significantly reduces gliotoxin biosynthesis.¹²⁹ Moreover, the disruption of the *hdaA* homolog in the endophytic fungus *Penicillium chrysogenum* Fes1701 has been demonstrated to significantly alter the secondary metabolic profile, resulting in

the emergence of alkaloids.¹³⁰ In this context, the BGC associated with meleagrin/roquefortine is upregulated, while the BGC for chrysogine is downregulated.

Mao et al. performed a targeted deletion of the *hdaA* gene, which encodes the histone H3 lysine 14 (H3K14) deacetylase, in the endophytic fungus *Calcarisporium arbuscula*.⁵⁸ This knockout resulted in a significant pleiotropic effect, activating and causing overexpression of 75% of the polyketide synthase (PKS) genes, 78% of the non-ribosomal peptide synthase (NRPS) genes, and 78% of the terpenoid synthase (TS) genes in *C. arbuscula*. These findings indicate that HdaA functions as a global suppressor of BGCs under axenic conditions.⁵⁸ Chemical analyses of the $\Delta hdaA$ mutant yielded two novel cyclic peptides (Figure 11), arbumycin and arbumelin (**53** and **54**), a new diterpenoid arbuscullic acid A (**55**), a new meroterpenoid arbuscullic acid B (**56**), and twelve additional novel diterpenoids (**57-68**), alongside various known polyketides.^{58,131}

The targeted deletion of *PfHdaA* in the endophytic fungus *Pestalotiopsis fici*, a homolog of *A. fumigatus HdaA*, significantly augmented the production of polyketides.¹³² This study led to the isolation of a novel polyketide (Figure 12), pestaloficiol W (**69**), alongside eleven new macrodiolides, ficiolides A–K (**70-80**), with ficiolide K (**80**) featuring a rare 1,6-anhydro-pyranose unit.¹³² Subsequent genetic deletion of *PfptaA*, which encodes the biosynthetic pathway for the principal compounds pestheic acid and its derivatives in *P. fici*, resulted in the construction of a $\Delta PfptaA\Delta PfhdaA$ strain.¹³³ This mutant exhibited a marked alteration in its metabolic profile, yielding a novel compound, pestaloficiol X (**81**, Figure 11).

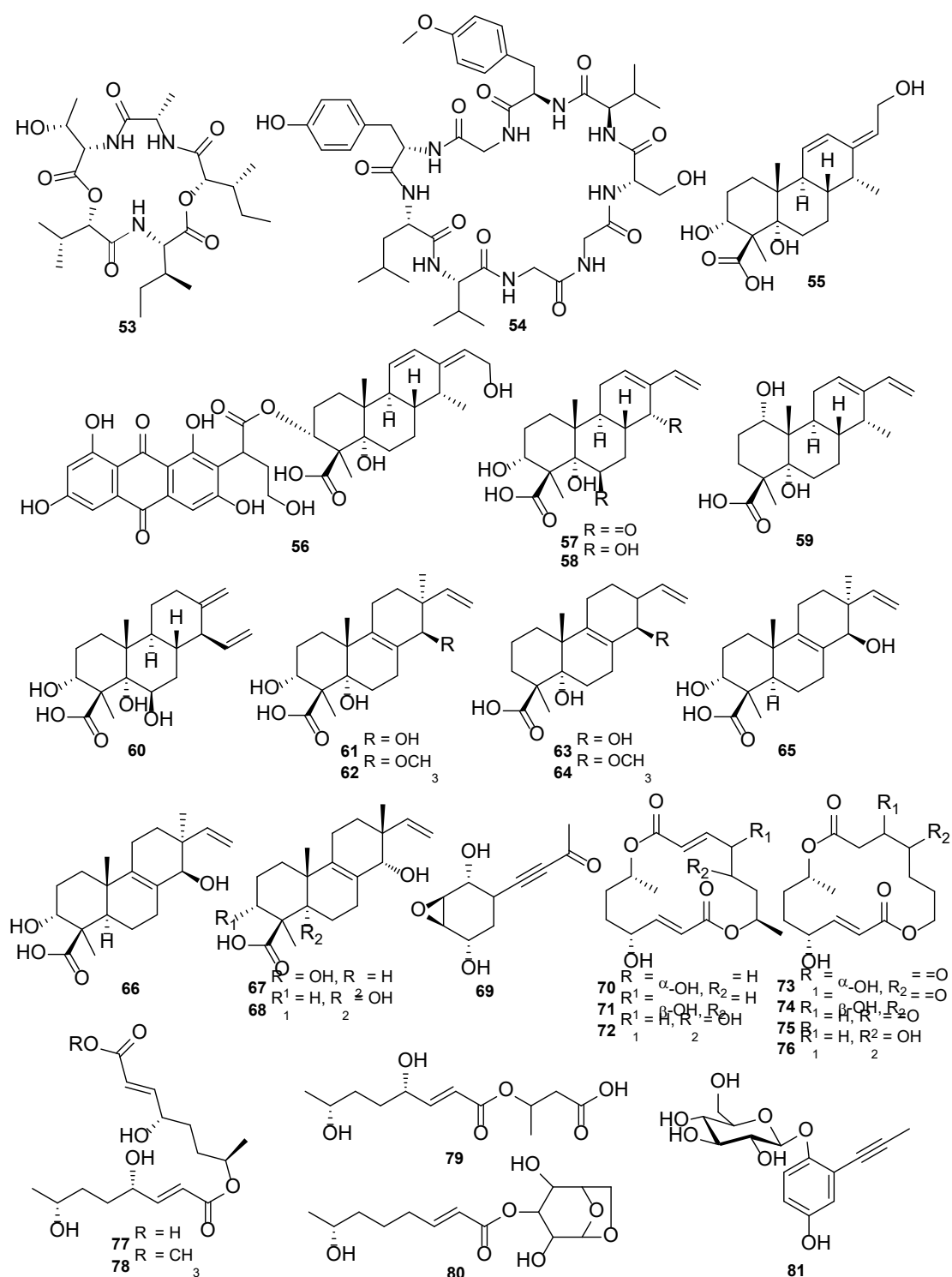


Figure 11. Chemical structures of compounds **53-81** induced by deleting epigenetic erasers (HDACs and HDMTs) in endophytic fungi

8.2 Writers (HATs and HKMTs)

Wang et al. adopted a histone acetyltransferase (HAT) overexpression strategy to activate silent BGCs in their study.¹³⁴ Focusing on the endophytic

fungus *Chaetomium globosum*, they utilized a *Trpc* promoter to enhance the expression of CgHAT, successfully upregulating a cryptic azaphilone gene cluster. This led to the novel isolation of a pair of atropisomeric dimeric bis-spiro-azaphilones, designated cochliodones A and B (**82** and **83**, [Figure 12](#)), from *C. globosum* CBS148.51.¹³⁴ Histone H3 lysine 4 (H3K4) methylation mediated by the histone methyltransferase CclA is extensively characterized in its role in regulating secondary metabolism in fungi.²² Disruption of CclA or its homologs typically results in the activation of silent secondary metabolite gene clusters.²² Research conducted by the O'Connell group has established that CclA is indispensable for global H3K4 trimethylation in the fungus *Colletotrichum higginsianum*, thereby influencing both fungal growth and secondary metabolic pathways.¹³⁵ The deletion of CclA led to a pronounced upregulation of three distinct BGCs, which are implicated in the biosynthesis of five novel compounds ([Figure 12](#)): colletorin D (**84**), colletorin D acid (**85**), higginsianin C (**86**), 13-epi-higginsianin C (**87**), and sclerosporide (**88**).¹³⁵ Similarly, targeted knockout of PfCclA in the endophytic fungus *Pestalotiopsis fici*—a homologous protein to *A. nidulans* CclA—significantly boosted secondary metabolite production, resulting in the identification of three new polyketides, designated pestaloficiols T–V (**89-91**, [Figure 12](#)).¹³²

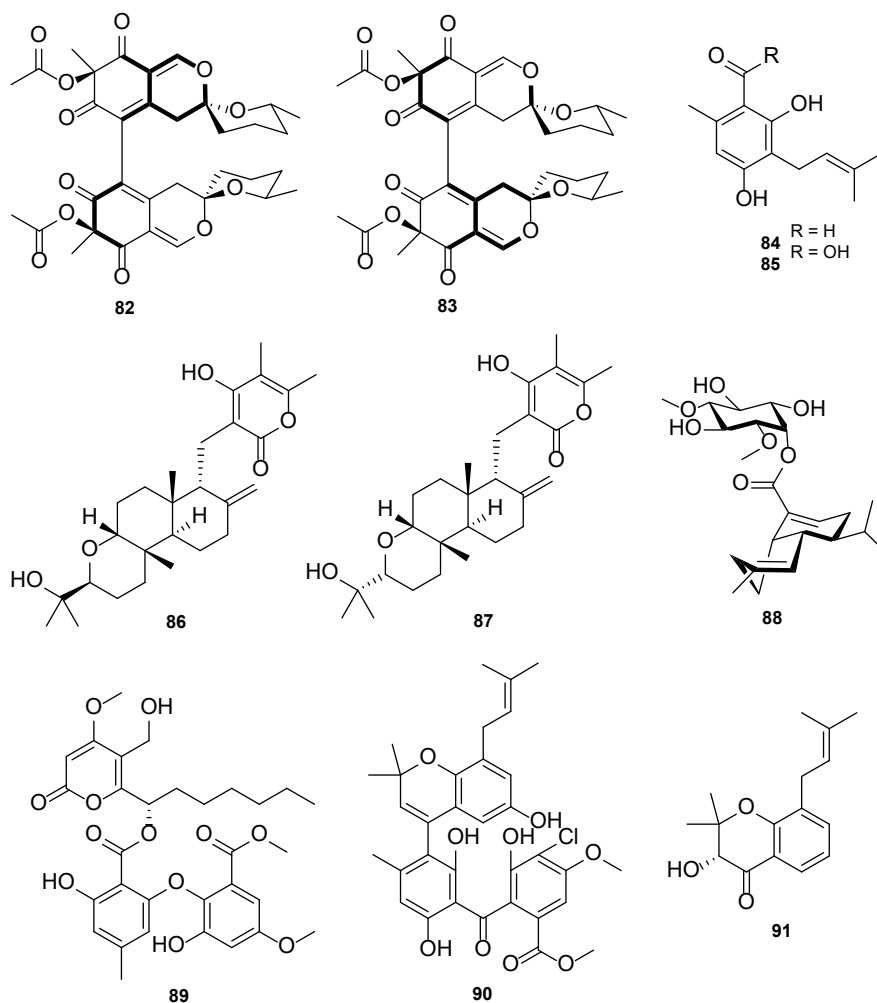


Figure 12. Chemical structures of compounds **82-91** induced by the overexpression or deletion of epigenetic writers (HATs and HKMTs) in fungi, including endophytic fungi.

8.3 Readers (SntB)

SntB is an essential epigenetic reader contributing to various facets of fungal physiology, including growth, development, virulence, and secondary metabolite production.¹³⁶ The pioneering deletion of the epigenetic reader StnB was executed and validated by Pfannenstiel et al.¹³⁷ This study established StnB as a global regulator of secondary metabolism, modulating histone modifications such as H3K9 and H3K14 acetylation, along with H3K4 trimethylation in both *Aspergillus nidulans* and *Aspergillus flavus*.¹³⁸ The $\Delta stnB$ mutant exhibited a loss of biosynthetic capability for several secondary metabolites, including aflavarin, aflatoxin B1, asparasone A, and aflatrem.

Conversely, the StnB deletion strain was associated with elevated production of ditryptophenaline and leporin B.¹³⁸ Additionally, the loss of SntB in *A. flavus* triggered the biosynthesis of two previously cryptic cyclic hexa-depsipeptides, aspergillicins A and F (**92** and **93**, Figure 13).¹³⁹ Furthermore, SntB has been shown to positively influence the expression of three key global regulators of virulence and secondary metabolism—LaeA, CreA, and PacC—thereby enhancing the production of toxic compounds such as patulin and citrinin in *Penicillium expansum*.¹³⁶

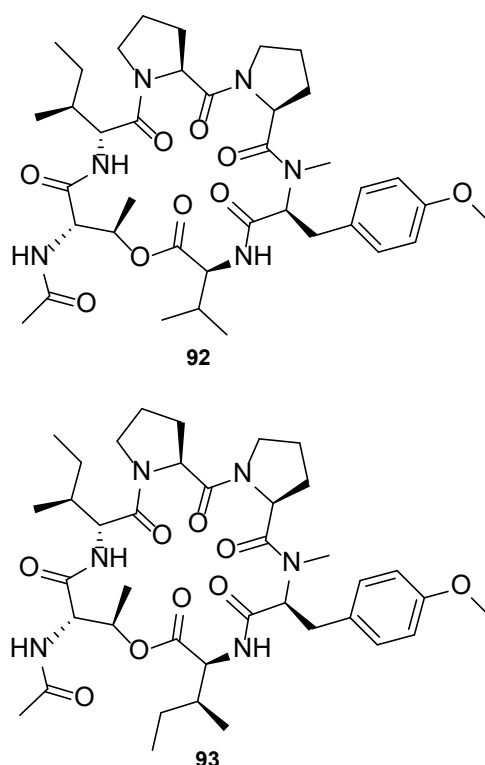


Figure 13. Chemical structures of compounds **92** and **93** induced by deleting an epigenetic reader (SntB) in *A. flavus*.

8.4 The integrated strategy

Recent studies have highlighted the diversity of epigenetic marks present in fungi; however, comprehensive investigations that integrate these modifications remain limited. A significant challenge in manipulating epigenetic regulators stems from the complex interplay of numerous modifications that can act either synergistically or antagonistically in gene expression regulation. For

instance, in *Neurospora crassa*, the HDAC complex HCHC collaborates with the DNMT complex DIM-2-HP1 to establish and maintain heterochromatin.¹⁴⁰ By concurrently inactivating multiple epigenetic regulators, researchers can more effectively shift global transcriptional landscapes, enhancing the activation of previously silent BGCs and enabling the exploration of novel biosynthetic pathways that may not be accessible through modification of a single regulatory axis.¹⁴¹ In a study aimed at activating silent BGCs in *Aspergillus nidulans*, Zhao et al. employed a combinatorial approach to inactivate three key epigenetic regulators, viz. HdaA, CclA, and ClrD.¹⁴¹ This strategy led to the isolation of three novel hybrid compounds (**94**, **95**, and **96**, Figure 14), which were identified as products of the interaction between the cichorine and polyamine biosynthetic pathways.

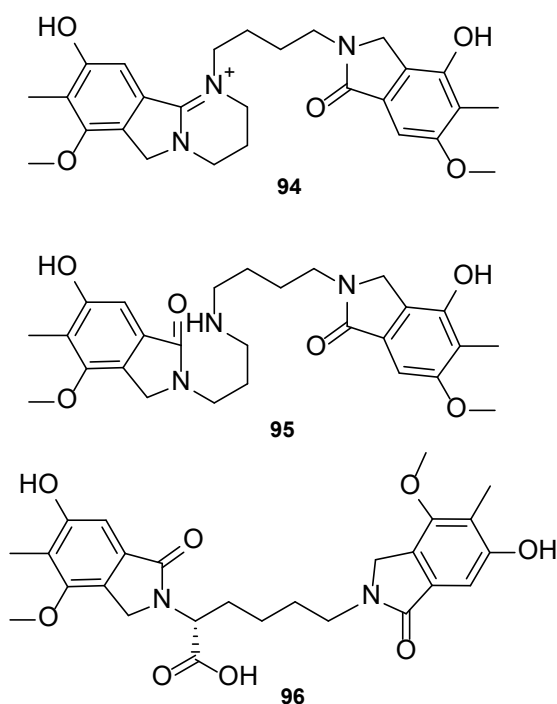


Figure 14. Chemical structures of compounds **94-96** induced by the simultaneous deletion of three epigenetic regulators (HdaA, CclA, and ClrD) in *A. nidulans*.

9. CRISPR/dCas9-mediated epigenetic modification

While the deletion and overexpression of epigenetic factors have yielded

some beneficial outcomes, such interventions tend to be non-selective and impact the genome on a global scale. The application of the Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR associated protein 9 (CRISPR/Cas9) technology can be directed to specific gene loci, enabling targeted double-stranded DNA cleavage.¹⁴² Researchers have developed a catalytically inactive variant known as dead Cas9 (dCas9) by engineering mutations in two essential nuclease structural domains. Although dCas9 cannot cleave DNA, it retains the ability to bind to a specific DNA sequence in conjunction with a small guide RNA (sgRNA).¹⁴³ When dCas9 is fused to various epigenetic modification domains or enzymatic effectors, it serves as a versatile DNA-binding platform for modulating gene expression. DNA-binding platform for modulating gene expression. This approach allows for the precise installation or removal of epigenetic markers, facilitating the targeted reprogramming of gene expression in eukaryotic systems, and can ultimately enhance secondary metabolite yields in endophytic fungal systems.

Hilton et al. demonstrated that the fusion of the p300 core domain, a human histone acetyltransferase, with dCas9, led to efficient acetylation of H3K27 at designated genomic loci in mammalian cells.¹⁴⁴ This modification corresponded with significant transcriptional activation of genes associated with promoters, proximal enhancers, and distal enhancers. Likewise, various epigenetic regulators have been successfully coupled with CRISPR/dCas9 for targeted transcriptional modulation in mammalian cells, including the histone deacetylase HDAC3,¹⁴⁵ histone methyltransferases DOT1L and PRDM9,¹⁴⁶ and histone demethylase LSD1.¹⁴⁷ Additionally, DNA methyltransferase DNMT3A and its murine homolog Dnmt3a,^{148,149} as well as the DNA demethylase Tet1,¹⁴⁹ have been employed in this context. The versatility of fusing varied epigenetic effector domains to dCas9 for gene regulation has also been demonstrated in organisms such as *Plasmodium falciparum*,¹⁵⁰ *Bombyx mori*,¹⁵¹ and various plant species.¹⁵²

CRISPR/dCas9-based tools, including CRISPR transcriptional and epigenetic regulators, have also been developed to manipulate BGCs in various fungi and bacteria.¹⁵³ Transcriptional modulation using CRISPR typically employs dCas9 and dCas12a through CRISPR-based activation (CRISPRa) and inhibition (CRISPRi), allowing for the targeted delivery of effector proteins to specific regulatory sequences.¹⁵³ A notable application of a dCas9 activator system by fusing activator proteins with dCas9 was demonstrated in myxobacteria, where it was used to enhance the expression of exogenous epothilone biosynthetic genes in *Myxococcus xanthus*.¹⁵⁴ In parallel, targeted epigenome editing is achievable by fusing dCas proteins with epigenetic effectors.¹⁵³ For instance, to spur the production of secondary metabolites in *Aspergillus niger*, Li et al. devised a CRISPR/dCas9-mediated system that enables precise control over gene expression.¹⁵⁵ They constructed fusions of various epigenetic regulators with CRISPR/dCas9 to regulate secondary metabolic genes, such as *breF*. The histone acetyltransferase p300-dCas9 fusion could target the promoter region adjacent to the ATG of the *breF* gene, subsequently activating its transcription. Additionally, they deployed endogenous histone modification effectors fused to dCas9 upstream of *breF*'s ATG, confirming that the histone acetylase GcnE-dCas9, along with the histone deacetylases HosA-dCas9 and RdpA-dCas9, effectively repressed *breF* transcription.¹⁵⁵ Although CRISPR/dCas9 offers higher specificity than its active Cas9 counterpart,¹⁴² off-target modifications at unintended loci can still occur.¹⁵⁶ For example, the HosA-dCas9 system, initially intended to specifically target the *fwnA* gene and enhance melanin biosynthesis in *A. niger*, inadvertently increased the transcription of the pigment gene *ovIA*.¹⁵⁵

10. Biological and nature-inspired modulation of endophytic fungal epigenetic machinery

As mentioned above, certain chemicals serve as epigenetic modulators,

significantly impacting the secondary metabolite production in endophytic fungi. The role of environmental fungi in mediating interactions with other microorganisms through epigenetic alterations is becoming increasingly apparent in activating a diverse array of secondary metabolic pathways.^{157,158} This phenomenon is particularly salient in endophytic fungi that form symbiotic relationships with plants, exemplified by the associations between *Epichloë* spp. and grasses. In these systems, the BGCs are often silent or exhibit minimal expression in axenic cultures but demonstrate robust expression within their plant hosts.^{33,159-161} Compounding this complexity, the endophytic fungus *Epichloë festucae* has been shown to facilitate the increased infection rate of the pathogenic fungus *Claviceps purpurea* in seeds of *Festuca rubra* (red fescue).¹⁶² This interaction subsequently drives the production of Claviceps alkaloids, which are toxic to herbivorous animals and serve to mitigate herbivore pressures.¹⁶² Root fungal endophytes have also demonstrated significant inter-kingdom synergistic effects with plant microbiota against aggressive soil-borne pathogens.¹⁶³

Given the extensive and often intricate interactions between endophytic fungi and their host plants, as well as the multifaceted interspecies relationships at play, it is plausible to consider that specific compounds, signaling mechanisms from host plants, natural products from associated organisms, and even biological agents within similar ecosystems could exert epigenetic influences on endophytic fungi. Although the underlying epigenetic effects of these natural compounds and organisms on endophytic fungi are not fully elucidated, such nature-derived modulations have the potential to activate BGCs that remain dormant or insufficiently expressed in controlled laboratory environments.

Taxol® (paclitaxel, **97**, Figure 15) is recognized as one of the most significant plant-derived anticancer agents in the pharmacological landscape. In 1993, Stierle et al. first described the production of compound **97** by the

endophytic fungus *Taxomyces andreanae* (currently reclassified as *Ceriporiopsis andreanae*¹⁶⁴), which was isolated from *Taxus brevifolia*.¹⁶⁵ This seminal finding has generated over 1800 citations in the Web of Science and has spurred a multitude of subsequent investigations into other endophytes exhibiting similar biosynthetic capabilities. However, recent metabolic analyses and functional genomics approaches aimed at verifying the potential of *C. andreanae* for producing compound **97** have yielded disappointing outcomes.^{164,166-168} The sustained production of compound **106** from various endophytic strains have also been attempted but remains elusive, primarily due to reduced yields linked to extensive subculturing and storage practices.¹⁶⁹

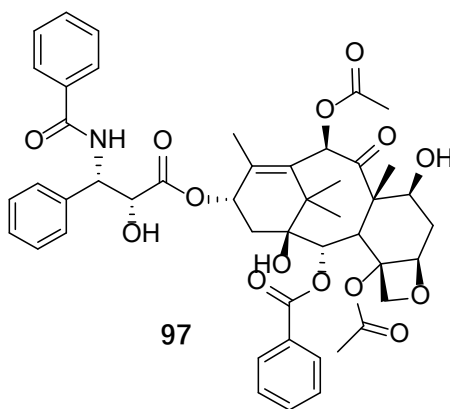


Figure 15. Chemical structures of paclitaxel (**97**), which could be induced by the biological and nature-inspired modulation of endophytic fungal epigenetic machinery.

El-Sayed et al. investigated novel fungal isolates exhibiting molecular and metabolic stability conducive to paclitaxel (**97**) biosynthesis. They discovered that incorporating surface-sterilized leaves from host plants significantly enhanced the yield of compound **97** by these endophytic fungi, whereas utilizing autoclaved leaves in the culture medium did not yield similar outcomes.¹⁷⁰⁻¹⁷² This finding underscores the hypothesis that proximal physical interactions between endogenous endophytes are crucial for eliciting the biosynthesis of compound **97**.¹¹ Further proteomic analyses have revealed a

marked upregulation of specific proteins, notably histones H2A and H2B, following these plant-fungi interactions,¹⁷³ implicating chromatin remodeling as a possible process in paclitaxel biosynthesis. Such closely associated physical interactions between microorganisms have been documented, leading to epigenetic regulatory mechanisms, including Saga/Ada-mediated histone acetylation that modulate fungal secondary metabolic pathways.^{157,158}

11. Epigenetic modulation at the plant-fungal interface

The intricate interplay between plants and their endophytic fungi underscores the significant role of epigenetic modifications in shaping these relationships. The epigenetic modulation of endophytes presents exciting potential, particularly for activating dormant BGCs, which facilitates the production of bioactive metabolites with medicinal value.¹⁷⁴ Additionally, these fungal endophytes and their metabolites can induce epigenetic alterations in their host plants when faced with various biotic and abiotic stressors.^{174,175} Such interactions enable endophytes to modulate plant gene expression and phenotypic traits via epigenetic changes, enhancing plant resilience to environmental challenges.¹⁷⁶ This understanding of plant-endophyte dynamics highlights the critical nature of their symbiotic relationships, revealing how endophyte colonization can lead to beneficial epigenetic modifications that fortify plant defenses against stress. By employing epigenetic manipulation, we can improve the efficiency of screening for bioactive compounds in plant systems while boosting plant resilience. This approach offers significant prospects for progress in agricultural biotechnology and medicinal science, paving the way for developing more resilient crop varieties and novel therapeutic strategies.

11.1 DNA methylations

DNA methylation is an evolutionarily conserved epigenetic modification

essential for gene expression regulation and maintaining genomic stability in plants.¹⁷⁷ Notably, this modification exhibits considerable divergence among different species and contexts.¹⁷⁷ The dynamic methylation and demethylation processes occur throughout the plant's life cycle, adapting to developmental cues and environmental stressors¹⁷⁹ and showing variability across plant tissues and species.¹⁸⁰ Both biotic and abiotic stressors have been shown to elicit alterations in DNA methylation profiles within plants¹⁸¹⁻¹⁸⁴.

Endophytic fungi establish complex interactions within plant tissues, enabling host plants to navigate biotic and abiotic stressors such as pathogens and drought. These fungal associates are known to influence the methylation dynamics of host plant genomes, which can enhance plant resilience and fitness under stress conditions. A study by Hubbard et al. illustrated that specific Ascomycota isolates, particularly the endophytic fungus SMCD 2206, significantly improved various growth metrics in wheat subjected to heat and drought stress¹⁸⁵. Notably, SMCD 2206 markedly lowered the hydrothermal time (HTT) requirement for germination while also enhancing the germination energy of wheat seeds under both stress conditions.^{186,187} These findings demonstrate that colonization by endophytic fungi, specifically strain SMCD 2206, mitigates the susceptibility of wheat to heat and drought, thereby improving tolerance levels. Furthermore, the analysis revealed that this strain induced epigenetic alterations in the DNA methylation profiles of wheat under drought stress.¹⁸⁵ The methylation patterns in drought-stressed wheat seedlings colonized by SMCD 2206 closely resembled those of unstressed controls,¹⁸⁵ highlighting the critical role of plant-endophyte interactions. These results suggest that endophyte-mediated epigenetic modifications can potentially bolster the resilience of host plants in the face of environmental stressors.

Epichloë spp. as endophytic fungi play a significant role in the ecological environment and have important implications for grassland ecosystems and agricultural production. They are notable fungal symbionts associated with

Poöideae grasses, including perennial ryegrass (*Lolium perenne*), tall fescue, and meadow fescue.¹⁸⁸ The interactions between these fungi and their hosts can range from mutualistic to pathogenic, largely contingent upon their modes of reproduction: primarily vertical transmission via seeds or horizontal transmission through ascospores.¹⁸⁹ Research has demonstrated that the vertical transmission of *Epichloë* fungal endophytes through seeds is significantly influenced by the genetic background of the host.¹⁹⁰ Seed-transmitted endophytes exploit host-derived sugars, amino acids, and other metabolites for sustenance, and in exchange, they confer protection against various biotic and abiotic stressors.^{191,192} This protection is largely mediated through the biosynthesis of specific bioactive alkaloids within the plant system. The metabolic interactions of these endophytes enhance the performance and resilience of pastures. Key bioactive alkaloids produced by wild-type *Epichloë* endophytes (also known as standard or common-toxic endophytes) include ergot alkaloids and indole diterpenes,¹⁹³ with ergovaline and lolitrem B being the principal terminal compounds. These metabolites exhibit toxicity towards herbivorous insects and mammals, presenting significant challenges for the pastoral sector.^{27,28} Conversely, safe-to-consume *Epichloë* endophytes have been selectively developed that offer bio-protective advantages, contributing to improved pasture persistence and productivity. These variants have been successfully commercialized and adopted in agricultural practices across the USA, Australia, and New Zealand.^{189,194}

The mutualistic association between *L. perenne* and *Epichloë* sp. AR37 is among the most effective interactions, yielding epoxy-janthitrem indole diterpenes that exhibit toxicity towards insects while presenting a lower risk to livestock.¹⁹⁵ Forte et al. demonstrated that the long-term establishment of *Epichloë* sp. AR37 within *L. perenne* led to global hypomethylation across multiple genomic features, with a pronounced effect in intergenic regions and CHH methylation contexts.¹⁹⁶ This hypomethylation phenomenon was also

evident across successive plant generations, suggesting a role for the endophyte in promoting this multi-generational complex hypomethylation process.¹⁹⁷ Furthermore, under drought stress conditions, *Epichloë* sp. AR37 markedly altered DNA methylation patterns in *L. perenne*.²¹⁶

11.1 Histone and chromatin modifications

Histone lysine methylation serves as a critical epigenetic modification in the regulation of transcription, with distinct methylation marks indicating either active (H3K4me2/3) or repressive (H3K9me2/3 and H3K27me2/3) chromatin states.^{26,197} Recent findings by Chujo and Scott highlight the importance of H3K9me3 and H3K27me3 trimethylation states in the chromatin of *Epichloë festucae*, which play a pivotal role in the symbiotic expression of bioprotective alkaloids.³¹ In plant-infected tissues, a marked reduction in the levels of H3K9me3 and H3K27me3 was observed at the loci responsible for lolitrems (BGC: *ltm*; *LTM* locus located on chromosome one) and ergot alkaloids (BGC: *eas*; *EAS* locus located on chromosome three) when compared to axenic cultures.^{31,198} The targeted deletion of H3K9 and H3K27 methyltransferases, encoded by the *clrD* and *ezhB* genes, respectively, resulted in the derepression of *ltm* and *eas* gene expression under non-symbiotic conditions, with this effect being further amplified in a double deletion mutant.³¹ This alteration in histone methylation status is essential not only for controlling alkaloid biosynthesis but also for the endophytic fungus's capacity to establish a mutualistic association with its grass host.³¹ Notably, the *E. festucae* $\Delta clrD$ mutant exhibited a pronounced growth deficiency in culture and failed to colonize *L. perenne* seedlings, emphasizing that the methylation mark influenced by $\Delta clrD$ is critical for the expression of genes vital to host cell interaction and colonization processes.

Subsequent investigations by Chujo et al. into the high-level expression of cluster genes *in planta* in response to chromatin remodeling have highlighted

the pivotal role of heterochromatin 1 protein (HepA) in mediating the chromatin landscape of sub-telomeric secondary metabolite genes, particularly in the context of symbiosis-specific regulation.¹⁹⁹ The deletion of HepA led to the derepression of *ltm* and *eas* gene expression under axenic culture conditions. Furthermore, when $\Delta hepA$ mutants were introduced to *L. perenne* seedlings, they exhibited a pronounced host interaction phenotype with diminished tiller length but an enhanced tiller count. This was also accompanied by reduced *ltm* gene expression and a loss of the capacity to produce lolitrems.¹⁹⁹

11.3 Other modulations

RNA interference (RNAi) represents a fundamental and conserved gene regulatory pathway in eukaryotic organisms, initiated by small RNAs (sRNAs) ranging from approximately 20 to 25 nucleotides in length. Recent work by Dalakouras et al. has elucidated the core components of the RNAi machinery in the *Fusarium solani* strain K, a beneficial root endophyte associated with *Nicotiana benthamiana*.²⁰⁰ Utilizing an engineered RNAi sensor system, they demonstrated that *F. solani* strain K is capable of delivering sRNA signals (including siRNAs and/or miRNAs) to *N. benthamiana*, which facilitates systemic gene silencing and induces epigenetic alterations.²⁰⁰ This underscores the potential role of RNAi-mediated communication between endophytes and their host plants in enhancing plant performance, particularly in the context of specific microbial associations. Moreover, the macrodomain gene *PpMACRO2* has been identified as prevalent in bryophytes yet lacking in vascular plants. It is hypothesized that bryophytes may have acquired *PpMACRO2* from mycorrhizal fungal interactions.²⁰¹ This gene is implicated in histone modification via ADP-ribosylation, instigating epigenetic modifications within the moss *Physcomitrella patens*.²⁰¹ These insights emphasize the importance of fungal symbioses and the role of ancestral gene sets in shaping the evolutionary trajectory of terrestrial plants during their initial colonization of land.

Increased in Bonsai Methylation 1 (IBM1) functions as a H3K9 demethylase in *Arabidopsis*.²⁰² The disruption of IBM1 leads to an elevation in genome-wide H3K9me2 levels. Investigations utilizing 16S rRNA gene sequencing have shown that impairment of this histone demethylase significantly modifies root-associated microbial communities within both the rhizosphere and endosphere.²⁰² The resultant alterations in plant-microbe interactions are closely linked to the plant autoimmunity observed in IBM1 mutants, as indicated by transcriptomic analyses highlighting salicylic acid-mediated defense pathways, systemic acquired resistance mechanisms, and increased synthesis of the defense metabolite camalexin.

Phenazine-1-carboxamide, a secondary metabolite produced by the bacterium *Pseudomonas piscium*, isolated from the wheat head microbiome, was shown to modulate the activity of FgGcn5, a histone acetyltransferase within the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex in the wheat pathogen *Fusarium graminearum*.²⁰³ This interaction led to specific deregulation of histone acetylation, notably affecting residues such as H2BK11, H3K14, H3K18, and H3K27. The resultant epigenetic modifications inhibited fungal growth, reduced virulence, and decreased mycotoxin production.²⁰³ These findings underscore the potential of antagonistic bacteria to mitigate the pathogenicity of plant-pathogenic fungi through the strategic alteration of histone modifications.

12. Artificial Intelligence (AI) in epigenetic modulation

Recent advancements in understanding the epigenetic mechanisms governing fungi, particularly endophytes, have highlighted their intricate interactions with host organisms. However, our current frameworks for cataloging the myriad epigenetic markers and their complex interrelations remain inadequate. Concurrently, the volume of multifaceted datasets—encompassing genomics, epigenomics, transcriptomics, proteomics, and

metabolomics—has surged.²⁰⁴ The recent progress in artificial intelligence (AI), particularly through machine learning (ML) and deep learning (DL) methodologies, has markedly transformed the analysis of epigenetic modifications, equipping researchers with powerful tools to decipher complex epigenomic datasets, especially in fungal systems.²⁰⁵⁻²⁰⁷ Numerous AI-driven methodologies for modeling and anticipating epigenomic marks, predominantly concerning humans, mice, and plants, have been extensively reviewed.^{205,206} This efficacy can be attributed to the availability of large and heterogeneous datasets for these organisms, including epigenomic datasets for various human primary cells and tissues in the Reference Epigenome Mapping Centers (REMCs).²⁰⁸ DeepChrome, a convolutional neural network that leverages DL, excels at classifying gene expression levels across 56 distinct human cell types using histone modification data as input.²⁰⁹ It also uncovers complex combinatorial relationships among these histone marks that govern gene regulation. Recently, Chromoformer, a transformer-based architecture that incorporates three-dimensional chromatin conformation, has surpassed existing DL models in gene expression prediction by capitalizing on human epigenetic features.²¹⁰ eDICE, an attention-based deep learning model, was developed to impute missing epigenomic tracks by leveraging sets of existing data.²¹¹ It can predict individual-specific epigenomic variations, even in tissues that lack comprehensive mapping in personal epigenomes. Wang et al. introduced a deep learning approach called SMEP, designed to mine epigenomic datasets from a variety of plant species and facilitate whole-genome predictions of diverse epigenomic modifications.²¹² Notably, in datasets from the Japonica rice variety Nipponbare, SMEP achieved prediction accuracies exceeding 95% for 6mA, even when accounting for biotic stressors.

The application of AI in predicting epigenetic modifications and their consequent effects on gene expression in fungi is gaining traction.²⁰⁵ Techniques such as Hi-C, which employs chromosome conformation capture,

provide comprehensive genome-wide (contact) data on chromosomal interactions, essential for exploring the three-dimensional architecture of chromatin.²¹³ Notably, the analysis of Hi-C datasets has enabled the identification of a jet-like domain within the deoxynivalenol BGC of *Fusarium graminearum*, a domain linked to active transcriptional processes and histone acetylation facilitated by the acetyltransferase GCN5.²¹⁴ Several ML methodologies, including the unsupervised clustering approach ClusterTAD,²¹³ and DL techniques such as the convolutional neural network HiCNN,²¹⁵ as well as the SRHiC model based on ResNet and WDSR architectures,²¹⁶ have been developed utilizing Hi-C data. These AI-driven approaches not only facilitate the identification of specific chromosomal regions but also enhance the resolution of Hi-C datasets, thereby advancing our understanding of chromatin dynamics and gene regulation.

Cook et al. investigated the impact of physical and chemical characteristics of DNA on genome evolution in the fungus *Verticillium dahliae*, revealing notable incomplete methylation of repetitive elements specifically within Lineage-Specific (LS) genomic regions enriched with genes relevant to host adaptation.²¹⁷ Their chromatin analysis established a correlation with H3K27me3 modifications and accessible genomic DNA. To further explore these relationships, they employed multiple supervised ML models, including logistic regression (LR), stochastic gradient boosting (GBM), boosted classification trees (BCT), and random forest (RF).²¹⁷ These models identified a greater number of LS genomic regions than previously documented, a finding corroborated by orthogonal validation analyses. The results underscore the defining role of distinct chromatin profiles in shaping adaptive genomic landscapes, highlighting the interplay of epigenetic factors in organizing these functional domains.

6mA data, rich in epigenetic information, is crucial for various biological processes and has been extensively characterized in the yeast species

Saccharomyces cerevisiae.^{218,219} Traditional experimental methods have been complemented by ML models for the identification of methylation sites directly from DNA sequences. Among these, LR classifiers and support vector machines have been employed effectively.^{218,219} Qiang et al. introduced the 6mA predictor M6AMRFS, which leverages an eXtreme Gradient Boosting (XGBoost) algorithm tailored for *S. cerevisiae*.²²⁰ This model incorporates dinucleotide binary encoding along with local position-specific dinucleotide features as descriptors. Using a dataset derived from the TSm6A-DL study, which encompasses both DNA and RNA sequence data from *S. cerevisiae*, an RF model was also developed utilizing binary encoding for predicting 6mA sites.²²¹ However, this model demonstrated marginally inferior performance compared to M6AMRFS in accurately identifying 6mA locations. These ML approaches not only enhance our understanding of 6mA's biological roles but also shed light on the underlying mechanisms governing DNA modifications.

13. Conclusion

In conclusion, activating dormant or low-expression biosynthetic BGCs in endophytic fungi via epigenetic mechanisms presents significant opportunities for identifying and producing novel bioactive compounds. By modulating epigenetic factors such as histone acetylation, histone methylation, and DNA methylation, researchers can reveal the metabolic potential of these fungi, potentially leading to significant advancements in pharmacology and biotechnology. Adopting cutting-edge strategies—such as innovative chemical modulators, genetic engineering, and AI-enhanced predictive models—highlights the pivotal role of epigenetic regulation in tapping into the underexplored metabolic capabilities of fungal endophytes. The field of epigenetics provides a robust framework for advancing our understanding of plant-fungal interactions while facilitating the translation of research outcomes into actionable intellectual property. This includes leveraging endophytic fungi as microbial platforms for biotechnological production of pharmaceutically

relevant compounds.

14. Future prospects

Several critical areas warrant further exploration in the realm of fungal endophyte epigenetics. The regulatory networks governing epigenetic modifications in these organisms are notably intricate, and the precise interplay between various epigenetic alterations and their influence on gene expression remains inadequately characterized. Gaining insights into these mechanisms is imperative for the targeted and effective activation of BGCs, particularly those whose expression is restricted to plant hosts. Current epigenetic approaches aimed at reactivating silent or under-expressed BGCs frequently encounter off-target effects, which inhibit the effective mining of the metabolic potential harbored within endophytic fungi. Future investigations should focus on the development of more selective and robust strategies to enhance metabolite production. Presently, chemical epigenetic modifiers such as SAHA and 5-azacytidine are the most commonly utilized in the context of endophytic fungi. However, there exists an opportunity to harness additional epigenetic modulators that precisely target relevant epigenetic regulators known to impact cancer, thereby refining the manipulation of endophytic fungal epigenetic processes. Furthermore, the implications of epigenetic regulation on the symbiotic interactions between endophytes and their host plants in natural ecosystems remain largely unexplored. Advancing our understanding in this area could inform sustainable agricultural practices and yield new insights into the evolutionary dynamics and functional attributes of these symbiotic systems. The current framework for delineating the comprehensive landscape of epigenetic markers and their intricate interrelations remains underdeveloped. The inherent complexity and variability of epigenomic regulation in fungi necessitate advancing AI-driven methodologies capable of integrating diverse epigenomic datasets. These AI-based tools can effectively process the

extensive omics data derived from fungal endophytes, enabling the prediction of epigenetic modifications and their subsequent influences on gene expression.

Epigenetic modulation has significantly advanced the discovery of bioactive natural products derived from endophytic fungi, as illustrated in this review. However, it is crucial to note that many current investigations aimed at leveraging the metabolic capabilities of endophytic fungi via epigenetic approaches often overlook the intricate associations these fungi share with host plants and other organisms, including pathogens, pests, and external environmental factors, as well as endophytic microorganisms within their ecological contexts. To address this oversight, there is an urgent need for interdisciplinary research that can inform the strategic design of epigenetic interventions for the discovery of novel natural products. Particularly, CRISPR/dCas9-mediated epigenetic modifications present a compelling avenue for the precise manipulation of epigenetic marks that regulate plant–microbe interactions. By integrating natural product chemistry with chemical ecology, synthetic biology, molecular biology, and other relevant fields, the exploration of bioactive natural products in endophytic fungi through targeted epigenetic alterations will be significantly enhanced.

Looking forward, epigenetics as a scientific field is poised to transform the landscape of large-scale fungal endophyte biotechnology. This innovative approach could give rise to practical and sustainable methodologies for synthesizing pharmaceutical prodrugs and lead candidates derived from fungal endophytes, yielding new therapeutic agents. The stakeholders involved in this domain comprise a diverse array of participants, including researchers integrating the fields of chemistry and biology, entrepreneurs, as well as pharmaceutical and agricultural companies.

15. Data Availability

The current manuscript constitutes a review. It does not include any new,

unpublished, primary research results, software, or code, and no new data has been generated in this review. All referenced works have been duly cited in the references section of the manuscript.

16. Conflicts of interest

The authors declare no conflict of interest.

17. Acknowledgements

This work was supported in part by the National Natural Science Foundation of China (No. 81903494 and 41706077) and the Natural Science Foundation of Shandong Province (No. ZR2023MC119) to G.L. Research in the laboratory of S.K. is supported in part by Pavol Jozef Šafárik University in Košice, Slovakia.

18. References

1. D. J. Newman and G. M. Cragg, *J. Nat. Prod.*, 2020, **83**, 770-803.
2. D. Domingo-Fernández, Y. Gadiya, A. J. Preto, C. A. Krettler, S. Mubeen, A. Allen, D. Healey and V. Colluru, *J. Nat. Prod.*, 2024, **87**, 1844–1851.
3. S. Kusari, C. Hertweck and M. Spiteller, *Chem. Biol.*, 2012, **19**, 792-798.
4. R. X. Tan and W. X. Zou, *Nat. Prod. Rep.*, 2001, **18**, 448–459.
5. G. Strobel and B. Daisy, *Microbiol. Mol. Biol. Rev.*, 2003, **67**, 491-502.
6. E. M. Freeman, *Philos. Trans. R. Soc. Lond. B: Biol. Sci.*, 1904, **196**, 27-30.
7. H. Zheng, M. Qiao, J. Xu and Z. Yu, *Front. Fungal Biol.*, 2021, **2**, 692549.
8. C. J. Eaton, M. P. Cox and B. Scott, *Plant Sci.*, 2011, **180**, 190-195.
9. H. Lu, T. Wei, H. Lou, X. Shu and Q. Chen, *J. Fungi*, 2021, **7**, 719-740.
10. W.-X. Wang, S. Kusari and M. Spiteller, in *Fungal Applications in Sustainable Environmental Biotechnology*, 2016, Chapter 14, pp. 353-370.
11. S. Kusari, M. Lamshöft, P. Kusari, S. Gottfried, S. Zühlke, K. Louven, U. Hentschel, O. Kayser and M. Spiteller, *J. Nat. Prod.*, 2014, **77**, 2577-2584.
12. R. N. Kharwar, A. Mishra, S. K. Gond, A. Stierle and D. Stierle, *Nat. Prod. Rep.*, 2011, **28**, 1208-1228.
13. H.-S. Joo, S. T. Deyrup and S. H. Shim, *Phytochem. Rev.*, 2020, **20**, 543-568.
14. P. J. Rutledge and G. L. Challis, *Nat. Rev. Microbiol.*, 2015, **13**, 509-523.
15. H.-N. Lyu, H.-W. Liu, N. P. Keller and W.-B. Yin, *Nat. Prod. Rep.*, 2020,

- 37**, 6-16.
16. M. Sang, P. Feng, L.-P. Chi and W. Zhang, *Nat. Prod. Rep.*, 2024, **41**, 565-603.
 17. S. L. Berger, T. Kouzarides, R. Shiekhata and A. Shilatifard, *Gene. Dev.*, 2009, **23**, 781-783.
 18. C. Deans and K. A. Maggert, *Genetics*, 2015, **199**, 887-896.
 19. E. R. Gibney and C. M. Nolan, *Heredity*, 2010, **105**, 4-13.
 20. R. H. Cichewicz, *Nat. Prod. Rep.*, 2010, **27**, 11-22.
 21. F. L. Cherblanc, R. W. M. Davidson, P. Di Fruscia, N. Srimongkolpithak and M. J. Fuchter, *Nat. Prod. Rep.*, 2013, **30**, 605-624.
 22. B. T. Pfannenstiel and N. P. Keller, *Biotechnol. Adv.*, 2019, **37**, 107345.
 23. J. C. Nielsen, S. Grijseels, S. Prigent, B. Ji, J. Dainat, K. F. Nielsen, J. C. Frisvad, M. Workman and J. Nielsen, *Nat. Microbiol.*, 2017, **2**, 17044-17053.
 24. R. J. Cox and T. A. M. Gulder, *Nat. Prod. Rep.*, 2023, **40**, 7-8.
 25. N. P. Keller, *Nat. Rev. Microbiol.*, 2018, **17**, 167-180.
 26. J. Heitman, C. L. Schardl, C. A. Young, U. Hesse, S. G. Amyotte, K. Andreeva, P. J. Calie, D. J. Fleetwood, D. C. Haws, N. Moore, B. Oeser, D. G. Panaccione, K. K. Schweri, C. R. Voisey, M. L. Farman, J. W. Jaromczyk, B. A. Roe, D. M. O'Sullivan, B. Scott, P. Tudzynski, Z. An, E. G. Arnaoudova, C. T. Bullock, N. D. Charlton, L. Chen, M. Cox, R. D. Dinkins, S. Florea, A. E. Glenn, A. Gordon, U. Guldener, D. R. Harris, W. Hollin, J. Jaromczyk, R. D. Johnson, A. K. Khan, E. Leistner, A. Leuchtmann, C. Li, J. Liu, J. Liu, M. Liu, W. Mace, C. Machado, P. Nagabhyru, J. Pan, J. Schmid, K. Sugawara, U. Steiner, J. E. Takach, E. Tanaka, J. S. Webb, E. V. Wilson, J. L. Wiseman, R. Yoshida and Z. Zeng, *PLoS Genet.*, 2013, **9**, e1003323.
 27. P. Guerre, *Toxins*, 2015, **7**, 773-790.
 28. G. Philippe, *Toxins*, 2016, **8**, 47-63.
 29. C. Wallwey and S.-M. Li, *Nat. Prod. Rep.*, 2011, **28**, 496-510.
 30. D. Jakubczyk, J. Z. Cheng and S. E. O'Connor, *Nat. Prod. Rep.*, 2014, **31**, 1328-1338.
 31. T. Chujo and B. Scott, *Mol. Microbiol.*, 2014, **92**, 413-434.
 32. C. Young, C. Schardl, D. Panaccione, S. Florea, J. Takach, N. Charlton, N. Moore, J. Webb and J. Jaromczyk, *Toxins*, 2015, **7**, 1273-1302.
 33. D. J. Fleetwood, B. Scott, G. A. Lane, A. Tanaka and R. D. Johnson, *Appl. Environ. Microbiol.*, 2007, **73**, 2571-2579.
 34. D. Hudson, W. Mace, A. Popay, J. Jensen, C. McKenzie, C. Cameron and R. Johnson, *Toxins*, 2021, **13**, 76-86.
 35. V. Vikuk, C. A. Young, S. T. Lee, P. Nagabhyru, M. Krischke, M. J. Mueller, J. Krauss, *Appl. Environ. Microbiol.*, 2019, **98**, e00465-19.
 36. J. Wang, C. Machado, D. G. Panaccione, H.-F. Tsai and C. L. Schardl, *Fungal. Genet. Biol.*, 2004, **41**, 189-198.
 37. O. Rigbers and S.-M. Li, *J. Biol. Chem.*, 2008, **283**, 26859-26868.

38. N. Lorenz, J. Olšovská, M. Šulc and P. Tudzynski, *Appl. Environ. Microbiol.*, 2010, **76**, 1822-1830.
39. K. E. Goetz, C. M. Coyle, J. Z. Cheng, S. E. O'Connor and D. G. Panaccione, *Curr. Genet.*, 2011, **57**, 201-211.
40. C. Wallwey, M. Matuschek and S.-M. Li, *Arch. Microbiol.*, 2009, **192**, 127-134.
41. J. Z. Cheng, C. M. Coyle, D. G. Panaccione and S. E. O'Connor, *J. Am. Chem. Soc.*, 2010, **132**, 12835-12837.
42. T. Haarmann, I. Ortel, P. Tudzynski and U. Keller, *ChemBioChem*, 2006, **7**, 645-652.
43. D. G. Panaccione, R. D. Johnson, J. Wang, C. A. Young, P. Damrongsakool, B. Scott and C. L. Schard, *Proc. Natl. Acad. Sci.*, 2001, **98**, 12820–12825.
44. J. Havemann, D. Vogel, B. Loll and U. Keller, *Chem. Biol.*, 2014, **21**, 146-155.
45. S. I. S. Grewal and S. Jia, *Nat. Rev. Genet.*, 2007, **8**, 35-46.
46. D. V. Fyodorov, B.-R. Zhou, A. I. Skoultschi and Y. Bai, *Nat. Rev. Mol. Cell Biol.*, 2017, **19**, 192-206.
47. K. Maeshima, S. Iida, M. A. Shimazoe, S. Tamura and S. Ide, *Trends Cell Biol.*, 2024, **34**, 7-17.
48. R. K. McGinty and S. Tan, *Chem. Rev.*, 2014, **115**, 2255-2273.
49. G. Brosch, P. Loidl and S. Graessle, *FEMS Microbiol. Rev.*, 2008, **32**, 409-439.
50. N. Korolev, A. P. Lyubartsev and L. Nordenskiöld, *Sci. Rep.*, 2018, **8**, 1543-1557.
51. G. D. Bowman and M. G. Poirier, *Chem. Rev.*, 2014, **115**, 2274-2295.
52. M. Jaskelioff and C. L. Peterson, *Nat. Cell Biol.*, 2003, **5**, 395-399.
53. C. He, Z. Zhang, B. Li and S. Tian, *Microorganisms*, 2020, **8**, 227-241.
54. D. Luciano-Rosario, O. Barda, J. Tannous, D. Frawley, Ö. Bayram, D. Prusky, E. Sionov and N. P. Keller, *Fungal. Genet. Biol.*, 2023, **169**, 103837.
55. B. C. Taylor and N. L. Young, *Biochem. J.*, 2021, **478**, 511-532.
56. B. D. Strahl and C. D. Allis, *Nature*, 2000, **403**, 41-45.
57. J. W. Bok, Y.-M. Chiang, E. Szewczyk, Y. Reyes-Dominguez, A. D. Davidson, J. F. Sanchez, H.-C. Lo, K. Watanabe, J. Strauss, B. R. Oakley, C. C. C. Wang and N. P. Keller, *Nat. Chem. Biol.*, 2009, **5**, 462-464.
58. X. M. Mao, W. Xu, D. Li, W. B. Yin, Y. H. Chooi, Y. Q. Li, Y. Tang and Y. Hu, *Angew. Chem. Int. Ed.*, 2015, **54**, 7592-7596.
59. Y. Zheng, X. Huang and N. L. Kelleher, *Curr. Opin. Chem. Biol.*, 2016, **33**, 142-150.
60. R. Nuberini, G. Sigismondo and T. Bonaldi, *Epigenomics*, 2015, **8**, 429-445.
61. W. Lai, J. Mo, J. Yin, C. Lyu and H. Wang, *TrAC, Trends Anal. Chem.*, 2019, **110**, 173-182.
62. S. Boedi, Y. Reyes-Dominguez and J. Strauss, in *Fungal Secondary*

- Metabolism*, 2012, ch. Chapter 16, pp. 221-236.
63. Y. Zhang, J. Fan, J. Ye and L. Lu, *Mol. Microbiol.*, 2020, **115**, 1191-1206.
 64. G. Tang, J. Yuan, J. Wang, Y.-Z. Zhang, S.-S. Xie, H. Wang, Z. Tao, H. Liu, H. C. Kistler, Y. Zhao, C.-G. Duan, W. Liu, Z. Ma and Y. Chen, *Nucleic Acids Res.*, 2021, **49**, 10448-10464.
 65. X. Xu, Y. Chen, B. Li and S. Tian, *Microbiol. Spectrum*, 2023, **11**, e035045-22.
 66. A. Gacek-Matthews, L. M. Noble, C. Gruber, H. Berger, M. Sulyok, A. T. Marcos, J. Strauss and A. Andrianopoulos, *Mol. Microbiol.*, 2015, **96**, 839-860.
 67. H. Tang, B. Tian, A. R. Brasier, L. C. Sowers and K. Zhang, *Sci. Rep.*, 2016, **6**, 31537-31544.
 68. S. J. Mondo, R. O. Dannebaum, R. C. Kuo, K. B. Louie, A. J. Bewick, K. LaButti, S. Haridas, A. Kuo, A. Salamov, S. R. Ahrendt, R. Lau, B. P. Bowen, A. Lipzen, W. Sullivan, B. B. Andreopoulos, A. Clum, E. Lindquist, C. Daum, T. R. Northen, G. Kunde-Ramamoorthy, R. J. Schmitz, A. Gryganskyi, D. Culley, J. Magnuson, T. Y. James, M. A. O'Malley, J. E. Stajich, J. W. Spatafora, A. Visel and I. V. Grigoriev, *Nat. Genet.*, 2017, **49**, 964-968.
 69. P. J. Park, *Nat. Rev. Genet.*, 2009, **10**, 669-680.
 70. J. W. Bok, A. A. Soukup, E. Chadwick, Y. M. Chiang, C. C. C. Wang and N. P. Keller, *Mol. Microbiol.*, 2013, **89**, 963-974.
 71. J. Fischer, S. Y. Müller, T. Netzker, N. Jäger, A. Gacek-Matthews, K. Scherlach, M. C. Stroe, M. García-Altares, F. Pezzini, H. Schoeler, M. Reichelt, J. Gershenzon, M. K. C. Krespach, E. Shelest, V. Schroeckh, V. Valiante, T. Heinzel, C. Hertweck, J. Strauss and A. A. Brakhage, *eLife*, 2018, **7**, e40969.
 72. A. Pidroni, B. Faber, G. Brosch, I. Bauer and S. Graessle, *Front. Microbiol.*, 2018, **9**, 2212.
 73. L. K. Caesar, R. Montaser, N. P. Keller and N. L. Kelleher, *Nat. Prod. Rep.*, 2021, **38**, 2041-2065.
 74. C. Wu, Y. Yin, L. Zhu, Y. Zhang and Y.-Z. Li, *Drug Discovery Today*, 2022, **27**, 730-742.
 75. C.-Y. Wang, J.-Q. Hu, D.-G. Wang, Y.-Z. Li and C. Wu, *Nat. Prod. Rep.*, 2024, **41**, 905-934.
 76. G. Li, S. Kusari, C. Golz, H. Laatsch, C. Strohmam and M. Spiteller, *J. Nat. Prod.*, 2017, **80**, 983-988.
 77. C.-C. Gu, J. Zeng, X.-P. Peng, Y.-J. Sun, S.-Z. Yuan, X.-N. Wang, R.-S. Zhang, H.-X. Lou and G. Li, *J. Org. Chem.*, 2023, **88**, 3185-3192.
 78. J. Chen, Q. Liu, L. Zeng and X. Huang, *Front. Microbiol.*, 2020, **11**, 574736.
 79. X. Hou, L. Liu, Y. Li, P. Wang, X. Pan, D. Xu, D. Lai and L. Zhou, *Int. J. Mol. Sci.*, 2024, **26**, 25-49.
 80. J. Jeon, S. Kwon and Y.-H. Lee, *Plant. Pathol. J.*, 2014, **30**, 1-9.

81. L. Zhang, Y. Han, Q. Jiang, C. Wang, X. Chen, X. Li, F. Xu, Y. Jiang, Q. Wang and W. Xu, *Med. Res. Rev.*, 2014, **35**, 63-84.
82. Y. Núñez-Álvarez and M. Suelves, *FEBS. J.*, 2021, **289**, 2771-2792.
83. M. Freitag, *Annu. Rev. Microbiol.*, 2017, **71**, 413-439.
84. Y. Gong, S. Li, Y. Zhou, F. Chen and Y. Shao, *Int. J. Biol. Macromol.*, 2024, **255**, 128208.
85. C. Wang, J. Feng, Y. Chen, D. Li, L. Liu, Y. Wu, S. Zhang, S. Du and Y. Zhang, *Mitochondrion*, 2020, **51**, 88-96.
86. K. D. Robertson, *Nat. Rev. Genet.*, 2005, **6**, 597-610.
87. Y.-S. Nai, Y.-C. Huang, M.-R. Yen and P.-Y. Chen, *Front. Microbiol.*, 2021, **11**, 616922.
88. A. Jeltsch, W. Nellen and F. Lyko, *Trends Biochem. Sci.*, 2006, **31**, 306-308.
89. L. Zhang, Y. Mu, T. Li, J. Hu, H. Lin and L. Zhang, *Nucleic Acids Res.*, 2024, **52**, 3886-3895.
90. L. Zhang, W. Chen, L. M. Iyer, J. Hu, G. Wang, Y. Fu, M. Yu, Q. Dai, L. Aravind and C. He, *J. Am. Chem. Soc.*, 2014, **136**, 4801-4804.
91. Y. Mu, L. Zhang, J. Hu, J. Zhou, H.-W. Lin, C. He, H.-Z. Chen and L. Zhang, *Nat. Chem. Biol.*, 2022, **18**, 733-741.
92. A.-T. Hauser, D. Robaa and M. Jung, *Curr. Opin. Chem. Biol.*, 2018, **45**, 73-85.
93. M. Schiedel and S. J. Conway, *Curr. Opin. Chem. Biol.*, 2018, **45**, 166-178.
94. Y. Luan, J. Li, J. A. Bernatchez and R. Li, *J. Med. Chem.*, 2018, **62**, 3171-3183.
95. P. A. Marks and R. Breslow, *Nat. Biotechnol.*, 2007, **25**, 84-90.
96. H.-Z. Lee, V. E. Kwitkowski, P. L. Del Valle, M. S. Ricci, H. Saber, B. A. Habtemariam, J. Bullock, E. Bloomquist, Y. Li Shen, X.-H. Chen, J. Brown, N. Mehrotra, S. Dorff, R. Charlab, R. C. Kane, E. Kaminskas, R. Justice, A. T. Farrell and R. Pazdur, *Clin. Cancer. Res.*, 2015, **21**, 2666-2670.
97. M. L.-S. Y. Chen, D. N. Savoy, D. D. Billadeau, G. S. Dow and A. P. Kozikowski, *J. Med. Chem.*, 2008, **51**, 3437-3448.
98. Z. Chi, Y. Bai, J. Li, K. Wang, Y. Xu and Y. Luan, *Bioorg. Med. Chem. Lett.*, 2022, **71**, 128821.
99. C.-J. Gong, A.-H. Gao, Y.-M. Zhang, M.-B. Su, F. Chen, L. Sheng, Y.-B. Zhou, J.-Y. Li, J. Li and F.-J. Nan, *Eur. J. Med. Chem.*, 2016, **112**, 81-90.
100. A. Chidambaram, K. Sundararaju, R. K. Chidambaram, R. Subbiah, J. M. Jayaraj, K. Muthusamy and R. Vilwanathan, *J. Cell. Physiol.*, 2018, **233**, 5293-5309.
101. Z. Sun, C. Xu, J. Cheng, Z. Yang, T. Liu, B. Deng, X. Zhang, X. Peng and J. Chen, *J. Med. Chem.*, 2024, **67**, 13067-13088.
102. K. M. VanderMolen, W. McCulloch, C. J. Pearce and N. H. Oberlies, *J. Antibiot.*, 2011, **64**, 525-531.

103. L. Guillade, F. Sarno, H. Tarhonskaya, A. Nebbioso, S. Alvarez, A. Kawamura, C. J. Schofield, L. Altucci and Á. R. de Lera, *ChemMedChem*, 2018, **13**, 1949-1956.
104. C. A. Olsen and M. R. Ghadiri, *J. Med. Chem.*, 2009, **52**, 7836-7846.
105. G. N. Pandian, K. i. Shinohara, A. Ohtsuki, Y. Nakano, M. Masafumi, T. Bando, H. Nagase, Y. Yamada, A. Watanabe, N. Terada, S. Sato, H. Morinaga and H. Sugiyama, *ChemBioChem*, 2011, **12**, 2822-2828.
106. Z. Yu, J. Taniguchi, Y. Wei, G. N. Pandian, K. Hashiya, T. Bando and H. Sugiyama, *Eur. J. Med. Chem.*, 2017, **138**, 320-327.
107. E. Ballestar, Z. Huang, H. Jiang, X. Liu, Y. Chen, J. Wong, Q. Wang, W. Huang, T. Shi and J. Zhang, *PLoS One*, 2012, **7**, e39917.
108. Z. Li, T. Qin, Z. Li, X. Zhao, X. Zhang, T. Zhao, N. Yang, J. Miao, J. Ma and Z. Zhang, *Eur. J. Med. Chem.*, 2021, **225**, 113778.
109. H. Sirous, G. Campiani, S. Brogi, V. Calderone and G. Chemi, *Molecules*, 2020, **25**, 1952-1972.
110. H. Lv, W.-J. Li, P. Xu, J.-G. Tang, Y. Zheng, Y. Wan, Y. Lin, H. Wang and X.-N. Li, *Bioorg. Chem.*, 2024, **143**, 107093.
111. M. Xue, X. Hou, J. Fu, J. Zhang, J. Wang, Z. Zhao, D. Xu, D. Lai and L. Zhou, *J. Fungi*, 2023, **9**, 172-201.
112. X. Ma, X. Wei, S. Xing, X. Cheng, J. Lu, C. Yang, G. Zhao, Z. Dai, S. Hou, Y. Liu and D. Zhu, *Nat. Prod. Res.*, 2024, DOI: 10.1080/14786419.2024.2441496.
113. J. Sun, T. Awakawa, H. Noguchi and I. Abe, *Bioorg. Med. Chem. Lett.*, 2012, **22**, 6397-6400.
114. R. B. Williams, J. C. Henrikson, A. R. Hoover, A. E. Lee and R. H. Cichewicz, *Org. Biomol. Chem.*, 2008, **6**, 1857-2020.
115. H.-J. Chen, T. Awakawa, J.-Y. Sun, T. Wakimoto and I. Abe, *Nat. Prod. Bioprospect.*, 2013, **3**, 20-23.
116. A. Magotra, M. Kumar, M. Kushwaha, P. Awasthi, C. Raina, A. P. Gupta, B. A. Shah, S. G. Gandhi and A. Chaubey, *AMB Express*, 2017, **7**, 43-53.
117. J. R. Gubiani, E. M. K. Wijeratne, T. Shi, A. R. Araujo, A. E. Arnold, E. Chapman and A. A. L. Gunatilaka, *Bioorg. Med. Chem.*, 2017, **25**, 1860-1866.
118. M. Zhao, L.-Y. Yuan, D.-L. Guo, Y. Ye, Z.-M. Da-Wa, X.-L. Wang, F.-W. Ma, L. Chen, Y.-C. Gu, L.-S. Ding and Y. Zhou, *Phytochemistry*, 2018, **148**, 97-103.
119. G. E. Siless, G. L. Gallardo, M. A. Rodriguez, Y. A. Rincón, A. M. Godeas and G. M. Cabrera, *Chem. Biodivers.*, 2018, **15**, e1800133.
120. J. P. Makhwitine, H. M. Kumalo, S. I. Ndlovu and N. P. Mkhwanazi, *Microorganisms*, 2023, **11**, 1404-1422.
121. D. A. Adpressa, K. J. Stalheim, P. J. Proteau and S. Loesgen, *ACS Chem. Biol.*, 2017, **12**, 1842-1847.
122. C. Li, Y. Shao, W. Li, T. Yin, H. Li, H. Yan, X. Guo, B. Liu and B. He, *J.*

- Nat. Prod.*, 2022, **85**, 1486-1494.
123. X.-L. Yang, L. Huang and X.-L. Ruan, *J. Asian Nat. Prod. Res.*, 2014, **16**, 412-417.
 124. X.-Y. Cai, N. Li, Y. Li, R.-J. Zhang, P. Lin, L. Liu, H.-Y. Ye, W.-S. Wu and M. Zhao, *Phytochemistry*, 2022, **203**, 113426.
 125. C.-Y. Li, H.-T. Li, Y.-T. Shao, X.-Y. Guo, W. Li and T.-P. Yin, *Nat. Prod. Res.*, 2023, **38**, 581-588.
 126. N. Happyana, E. Hermawati, S. D. Ellita, L. D. Juliawaty, Y. M. Syah and E. H. Hakim, *Phytochem. Lett.*, 2024, **60**, 249-256.
 127. X.-L. Yang, T. Awakawa, T. Wakimoto and I. Abe, *Tetrahedron Lett.*, 2013, **54**, 5814-5817.
 128. E. K. Shwab, J. W. Bok, M. Tribus, J. Galehr, S. Graessle and N. P. Keller, *Eukaryotic Cell*, 2007, **6**, 1656-1664.
 129. I. Lee, J.-H. Oh, E. Keats Shwab, T. R. T. Dagenais, D. Andes and N. P. Keller, *Fungal. Genet. Biol.*, 2009, **46**, 782-790.
 130. Z. Ding, H. Zhou, X. Wang, H. Huang, H. Wang, R. Zhang, Z. Wang and J. Han, *Molecules*, 2020, **25**, 3657-3669.
 131. J. Bai, R. Mu, M. Dou, D. Yan, B. Liu, Q. Wei, J. Wan, Y. Tang and Y. Hu, *Acta Pharm. Sin. B*, 2018, **8**, 687-697.
 132. G. Wu, H. Zhou, P. Zhang, X. Wang, W. Li, W. Zhang, X. Liu, H.-W. Liu, N. P. Keller, Z. An and W.-B. Yin, *Org. Lett.*, 2016, **18**, 1832-1835.
 133. L. Yang, G. Wu, F. Meng, H. Ran, W. Yin, W. Li and X. Liu, *Int. J. Mol. Sci.*, 2022, **23**, 3686-3699.
 134. M.-H. Wang, T. Jiang, G. Ding, S.-B. Niu, X.-W. Wang, M. Yu, Y.-C. Gu, Q.-B. Zhang, J.-H. Chen, H.-M. Jia and Z.-M. Zou, *J. Antibiot.*, 2017, **70**, 801-804.
 135. J. F. Dallery, É. Adelin, G. Le Goff, S. Pigné, A. Auger, J. Ouazzani and R. J. O'Connell, *Mol. Plant Pathol.*, 2019, **20**, 831-842.
 136. J. Tannous, O. Barda, D. Luciano-Rosario, D. B. Prusky, E. Sionov and N. P. Keller, *Front. Microbiol.*, 2020, **11**, 610.
 137. B. T. Pfannenstiel, X. Zhao, J. Wortman, P. Wiemann, K. Throckmorton, J. E. Spraker, A. A. Soukup, X. Luo, D. L. Lindner, F. Y. Lim, B. P. Knox, B. Haas, G. J. Fischer, T. Choera, R. A. E. Butchko, J.-W. Bok, K. J. Affeldt, N. P. Keller, J. M. Palmer and B. G. Turgeon, *mBio*, 2017, **8**, e1246-e1217.
 138. B. T. Pfannenstiel, C. Greco, A. T. Sukowaty and N. P. Keller, *Fungal. Genet. Biol.*, 2018, **120**, 9-18.
 139. C. Greco, B. T. Pfannenstiel, J. C. Liu and N. P. Keller, *ACS Chem. Biol.*, 2019, **14**, 1121-1128.
 140. S. Honda, V. T. Bicocca, J. D. Gessaman, M. R. Rountree, A. Yokoyama, E. Y. Yu, J. M. L. Selker and E. U. Selker, *Proc. Natl. Acad. Sci.*, 2016, **113**, E6135-E6144.
 141. F. Zhao, C. Sun, Z. Liu, A. Cabrera, M. Escobar, S. Huang, Q. Yuan, Q. Nie, K. L. Luo, A. Lin, J. A. Vanegas, T. Zhu, I. B. Hilton and X. Gao, *J.*

- Am. Chem. Soc.*, 2022, **145**, 413-421.
142. T. Li, Y. Yang, H. Qi, W. Cui, L. Zhang, X. Fu, X. He, M. Liu, P.-f. Li and T. Yu, *Signal Transduction Targeted Ther.*, 2023, **8**, 36-29.
 143. L. S. Qi, Ma. H. Larson, L. A. Gilbert, J. A. Doudna, J. S. Weissman, A. P. Arkin and W. A. Lim, *Cell*, 2013, **152**, 1173-1183.
 144. I. B. Hilton, A. M. D'Ippolito, C. M. Vockley, P. I. Thakore, G. E. Crawford, T. E. Reddy and C. A. Gersbach, *Nat. Biotechnol.*, 2015, **33**, 510-517.
 145. D. Y. Kwon, Y.-T. Zhao, J. M. Lamonica and Z. Zhou, *Nat. Commun.*, 2017, **8**, 15315-15323.
 146. D. Cano-Rodriguez, R. A. F. Gjaltema, L. J. Jilderda, P. Jellema, J. Dokter-Fokkens, M. H. J. Ruijters and M. G. Rots, *Nat. Commun.*, 2016, **7**, 12284-12295.
 147. N. A. Kearns, H. Pham, B. Tabak, R. M. Genga, N. J. Silverstein, M. Garber and R. Maehr, *Nat. Methods*, 2015, **12**, 401-403.
 148. A. Vojta, P. Dobrinić, V. Tadić, L. Bočkor, P. Korać, B. Julg, M. Klasić and V. Zoldoš, *Nucleic Acids Res.*, 2016, **44**, 5615-5628.
 149. X. S. Liu, H. Wu, X. Ji, Y. Stelzer, X. Wu, S. Czauderna, J. Shu, D. Dadon, R. A. Young and R. Jaenisch, *Cell*, 2016, **167**, 233-247.
 150. B. Xiao, S. Yin, Y. Hu, M. Sun, J. Wei, Z. Huang, Y. Wen, X. Dai, H. Chen, J. Mu, L. Cui and L. Jiang, *Proc. Natl. Acad. Sci.*, 2018, **116**, 255-260.
 151. Y. Liu, S. Ma, J. Chang, T. Zhang, X. Chen, Y. Liang and Q. Xia, *Insect Biochem. Mol. Biol.*, 2019, **110**, 105-111.
 152. H. Candela, J. E. Lee, M. Neumann, D. I. Duro and M. Schmid, *PLoS One*, 2019, **14**, e0222778.
 153. C. Woodcraft, Y.-H. Chooi and I. Roux, *Nat. Prod. Rep.*, 2023, **40**, 158-173.
 154. R. Peng, Y. Wang, W.-w. Feng, X.-j. Yue, J.-h. Chen, X.-z. Hu, Z.-f. Li, D.-h. Sheng, Y.-m. Zhang and Y.-z. Li, *Microb. Cell Fact.*, 2018, **17**, 15-27.
 155. X. Li, L. Huang, L. Pan, B. Wang and L. Pan, *Microbiol. Res.*, 2021, **245**, 126694.
 156. C. Galonska, J. Charlton, A. L. Mattei, J. Donaghey, K. Clement, H. Gu, A. W. Mohammad, E. K. Stamenova, D. Cacchiarelli, S. Klages, B. Timmermann, T. Cantz, H. R. Schöler, A. Gnirke, M. J. Ziller and A. Meissner, *Nat. Commun.*, 2018, **9**, 597-606.
 157. H.-W. Nützmann, Y. Reyes-Dominguez, K. Scherlach, V. Schroeckh, F. Horn, A. Gacek, J. Schümann, C. Hertweck, J. Strauss and A. A. Brakhage, *Proc. Natl. Acad. Sci.*, 2011, **108**, 14282-14287.
 158. K. S. Volker Schroeckh, Hans-Wilhelm Nützmann, Ekaterina Shelest, Wolfgang Schmidt-Heck, Julia Schuemann, Karin Martin, Christian Hertweck and Axel A. Brakhage, *Proc. Natl. Acad. Sci.*, 2009, **104**, 14558-14563.
 159. C. A. Young, M. K. Bryant, M. J. Christensen, B. A. Tapper, G. T. Bryan and B. Scott, *Mol. Genet. Genomics*, 2005, **274**, 13-29.

160. A. Tanaka, B. A. Tapper, A. Popay, E. J. Parker and B. Scott, *Mol. Microbiol.*, 2005, **57**, 1036-1050.
161. C. A. Young, S. Felitti, K. Shields, G. Spangenberg, R. D. Johnson, G. T. Bryan, S. Saikia and B. Scott, *Fungal. Genet. Biol.*, 2006, **43**, 679-693.
162. M. Laihonon, K. Saikkonen, M. Helander, B. R. Vázquez de Aldana, I. Zabalgogezcoa and B. Fuchs, *Front. Microbiol.*, 2022, **12**, 786619.
163. L. K. Mahdi, S. Miyauchi, C. Uhlmann, R. Garrido-Oter, G. Langen, S. Wawra, Y. Niu, R. Guan, S. Robertson-Albertyn, D. Bulgarelli, J. E. Parker and A. Zuccaro, *ISME. J.*, 2022, **16**, 876-889.
164. T. Cheng, M. Kolařík, L. Quijada and M. Stadler, *IMA Fungus*, 2022, **13**, 17-25.
165. A. Stierle, G. Strobel and D. Stierle, *Science*, 1993, **260**, 214-216.
166. A. Staniek, H. Woerdenbag and O. Kayser, *Planta Med.*, 2009, **75**, 1561-1566.
167. U. Heinig, S. Scholz and S. Jennewein, *Fungal Diversity*, 2013, **60**, 161-170.
168. M. Stadler and M. Kolarik, *Fungal Biol. Rev.*, 2024, **49**, 100367.
169. A. Gamal, E. Fikry, N. Tawfeek, A. M. El-Shafae, A. S. A. El-Sayed and M. M. El-Domiaty, *Microb. Cell Fact.*, 2024, **23**, 78-97.
170. A. S. A. El-Sayed, D. M. I. Ali, M. A. Yassin, R. A. Zayed and G. S. Ali, *Process Biochem.*, 2019, **76**, 55-67.
171. A. S. A. El-Sayed, S. Safan, N. Z. Mohamed, L. Shaban, G. S. Ali and M. Z. Sitohy, *Process Biochem.*, 2018, **71**, 31-40.
172. A. S. A. El-Sayed, N. Z. Mohamed, S. Safan, M. A. Yassin, L. Shaban, A. A. Shindia, G. Shad Ali and M. Z. Sitohy, *Sci. Rep.*, 2019, **9**, 11534-11546.
173. A. S. A. El-Sayed, A. A. Shindia, A. AbouZeid, A. Koura, S. E. Hassanein and R. M. Ahmed, *Environ. Sci. Pollut. Res.*, 2021, **28**, 39866-39881.
174. L. Sena, E. Mica, G. Valè, P. Vaccino and N. Pecchioni, *Front. Plant Sci.*, 2024, **15**, 1349401.
175. S. Zeilinger, V. K. Gupta, T. E. S. Dahms, R. N. Silva, H. B. Singh, R. S. Upadhyay, E. V. Gomes, C. K.-M. Tsui, C. Nayak S and J. R. van der Meer, *FEMS Microbiol. Rev.*, 2016, **40**, 182-207.
176. P. Gallusci, D. R. Agius, P. N. Moschou, J. Dobránszki, E. Kaiserli and F. Martinelli, *Trends Plant Sci.*, 2023, **28**, 142-153.
177. H. Zhang, Z. Lang and J.-K. Zhu, *Nat. Rev. Mol. Cell Biol.*, 2018, **19**, 489-506.
178. S. Feng, S. J. Cokus, X. Zhang, P.-Y. Chen, M. Bostick, M. G. Goll, J. Hetzel, J. Jain, S. H. Strauss, M. E. Halpern, C. Ukomadu, K. C. Sadler, S. Pradhan, M. Pellegrini and S. E. Jacobsen, *Proc. Natl. Acad. Sci.*, 2010, **107**, 8689-8694.
179. A. S. Dubrovina, K. V. Kiselev and A. Weber, *Plant Biol.*, 2015, **18**, 185-196.
180. S. Varga, C. D. Soulsbury and P. Franken, *Plant Biol.*, 2018, **21**, 278-283.

181. W.-S. Wang, Y.-J. Pan, X.-Q. Zhao, D. Dwivedi, L.-H. Zhu, J. Ali, B.-Y. Fu and Z.-K. Li, *J. Exp. Bot.*, 2011, **62**, 1951-1960.
182. R. Aina, S. Sgorbati, A. Santagostino, M. Labra, A. Ghiani and S. Citterio, *Physiol. Plant.*, 2004, **121**, 472-480.
183. S. Varga and C. D. Soulsbury, *Biol. Lett.*, 2017, **13**, 20170407.
184. J. Vigneaud, A. Kohler, M. D. Sow, A. Delaunay, L. Fauchery, F. Guinet, C. Daviaud, K. W. Barry, K. Keymanesh, J. Johnson, V. Singan, I. Grigoriev, R. Fichot, D. Conde, M. Perales, J. Tost, F. M. Martin, I. Allona, S. H. Strauss, C. Veneault-Fourrey and S. Maury, *New Phytol.*, 2023, **238**, 2561-2577.
185. M. Hubbard, J. J. Germida and V. Vujanovic, *Can. J. Plant Sci.*, 2014, **94**, 223-234.
186. M. Hubbard, J. J. Germida and V. Vujanovic, *Botany*, 2012, **90**, 137-149.
187. M. Hubbard, J. J. Germida and V. Vujanovic, *J. Appl. Microbiol.*, 2014, **116**, 109-122.
188. S. D. Card, D. A. Bastías and J. R. Caradus, *Plants*, 2021, **10**, 10101997.
189. M. Kauppinen, K. Saikkonen, M. Helander, A. M. Pirttilä and P. R. Wäli, *Nat. Plants*, 2016, **2**, 15224-15231.
190. M. Gagic, M. J. Faville, W. Zhang, N. T. Forester, M. P. Rolston, R. D. Johnson, S. Ganesh, J. P. Koolaard, H. S. Easton, D. Hudson, L. J. Johnson, C. D. Moon and C. R. Voisey, *Front. Plant Sci.*, 2018, **9**, 1580.
191. P. R. Hardoim, L. S. van Overbeek, G. Berg, A. M. Pirttilä, S. Compant, A. Campisano, M. Döring and A. Sessitsch, *Microbiol. Mol. Biol. Rev.*, 2015, **79**, 293-320.
192. K. Lee, A. Missaoui, K. Mahmud, H. Presley and M. Lonnee, *Microorganisms*, 2021, **9**, 2186-2203.
193. D. E. Hume, D. Luo, G. M. Rennie, W. M. King, A. L. Taylor, M. J. Faville and K. N. Tozer, *Grassland Research*, 2024, **3**, 113-122.
194. L. J. Johnson, A. C. M. de Bonth, L. R. Briggs, J. R. Caradus, S. C. Finch, D. J. Fleetwood, L. R. Fletcher, D. E. Hume, R. D. Johnson, A. J. Popay, B. A. Tapper, W. R. Simpson, C. R. Voisey and S. D. Card, *Fungal Diversity*, 2013, **60**, 171-188.
195. F. P. Forte, J. Schmid, P. P. Dijkwel, I. Nagy, D. E. Hume, R. D. Johnson, W. R. Simpson, S. M. Monk, N. Zhang, T. Sehrish and T. Asp, *Front. Plant Sci.*, 2020, **11**, 570026.
196. F. P. Forte, M. Malinowska, I. Nagy, J. Schmid, P. Dijkwel, D. E. Hume, R. D. Johnson, W. R. Simpson and T. Asp, *Front. Plant Sci.*, 2023, **14**, 1258100.
197. T. S. Mikkelsen, M. Ku, D. B. Jaffe, B. Issac, E. Lieberman, G. Giannoukos, P. Alvarez, W. Brockman, T.-K. Kim, R. P. Koche, W. Lee, E. Mendenhall, A. O'Donovan, A. Presser, C. Russ, X. Xie, A. Meissner, M. Wernig, R. Jaenisch, C. Nusbaum, E. S. Lander and B. E. Bernstein, *Nature*, 2007, **448**, 553-560.
198. P. M. Richardson, D. J. Winter, A. R. D. Ganley, C. A. Young, I. Liachko,

- C. L. Schardl, P.-Y. Dupont, D. Berry, A. Ram, B. Scott and M. P. Cox, *PLos Genet.*, 2018, **14**, e1007467.
199. T. Chujo, Y. Lukito, C. J. Eaton, P.-Y. Dupont, L. J. Johnson, D. Winter, M. P. Cox and B. Scott, *Fungal. Genet. Biol.*, 2019, **125**, 71-83.
 200. A. Dalakouras, A. Katsaouni, M. Avramidou, E. Dadami, O. Tsiouri, S. Vasileiadis, A. Makris, M. E. Georgopoulou and K. K. Papadopoulou, *RNA Biol.*, 2022, **20**, 20-30.
 201. S. Wang, Y. Guan, Q. Wang, J. Zhao, G. Sun, X. Hu, M. P. Running, H. Sun and J. Huang, *Nat. Commun.*, 2020, **11**, 2030-2041.
 202. S. Lv, Y. Yang, G. Yu, L. Peng, S. Zheng, S. K. Singh, J. I. Vilchez, R. Kaushal, H. Zi, D. Yi, Y. Wang, S. Luo, X. Wu, Z. Zuo, W. Huang, R. Liu, J. Du, A. P. Macho, K. Tang and H. Zhang, *ISME. J.*, 2022, **16**, 2513-2524.
 203. Y. Chen, J. Wang, N. Yang, Z. Wen, X. Sun, Y. Chai and Z. Ma, *Nat. Commun.*, 2018, **9**, 3249-3263.
 204. G. Li, P. Lin, K. Wang, C.-C. Gu and S. Kusari, *Trends in Cancer*, 2022, **8**, 65-80.
 205. M. A. Machnicka and B. Wilczynski, in *Epigenetics of the Immune System*, 2020, DOI: 10.1016/b978-0-12-817964-2.00009-5, ch. 20, pp. 217-237.
 206. M. Hesami, M. Alizadeh, A. M. P. Jones and D. Torkamaneh, *Appl. Microbiol. Biotechnol.*, 2022, **106**, 3507-3530.
 207. D. Han, Q. Liu and W. Fan, *Expert Syst. Appl.*, 2018, **95**, 43-56.
 208. A. Kundaje, W. Meuleman, J. Ernst, M. Bilenky, A. Yen, A. Heravi-Moussavi, P. Kheradpour, Z. Zhang, J. Wang, M. J. Ziller, V. Amin, J. W. Whitaker, M. D. Schultz, L. D. Ward, A. Sarkar, G. Quon, R. S. Sandstrom, M. L. Eaton, Y.-C. Wu, A. R. Pfenning, X. Wang, M. Claussnitzer, Y. Liu, C. Coarfa, R. A. Harris, N. Shores, C. B. Epstein, E. Gjoneska, D. Leung, W. Xie, R. D. Hawkins, R. Lister, C. Hong, P. Gascard, A. J. Mungall, R. Moore, E. Chuah, A. Tam, T. K. Canfield, R. S. Hansen, R. Kaul, P. J. Sabo, M. S. Bansal, A. Carles, J. R. Dixon, K.-H. Farh, S. Feizi, R. Karlic, A.-R. Kim, A. Kulkarni, D. Li, R. Lowdon, G. Elliott, T. R. Mercer, S. J. Neph, V. Onuchic, P. Polak, N. Rajagopal, P. Ray, R. C. Sallari, K. T. Siebenthall, N. A. Sinnott-Armstrong, M. Stevens, R. E. Thurman, J. Wu, B. Zhang, X. Zhou, A. E. Beaudet, L. A. Boyer, P. L. De Jager, P. J. Farnham, S. J. Fisher, D. Haussler, S. J. M. Jones, W. Li, M. A. Marra, M. T. McManus, S. Sunyaev, J. A. Thomson, T. D. Tlsty, L.-H. Tsai, W. Wang, R. A. Waterland, M. Q. Zhang, L. H. Chadwick, B. E. Bernstein, J. F. Costello, J. R. Ecker, M. Hirst, A. Meissner, A. Milosavljevic, B. Ren, J. A. Stamatoyannopoulos, T. Wang and M. Kellis, *Nature*, 2015, **518**, 317-330.
 209. R. Singh, J. Lanchantin, G. Robins and Y. Qi, *Bioinformatics*, 2016, **32**, i639-i648.
 210. D. Lee, J. Yang and S. Kim, *Nat. Commun.*, 2022, **13**, 6678.

211. A. Hawkins-Hooker, G. Visonà, T. Narendra, M. Rojas-Carulla, B. Schölkopf and G. Schweikert, *Nat. Commun.*, 2023, **14**, 4750.
212. Y. Wang, P. Zhang, W. Guo, H. Liu, X. Li, Q. Zhang, Z. Du, G. Hu, X. Han, L. Pu, J. Tian and X. Gu, *New Phytol.*, 2021, **232**, 880-897.
213. O. Oluwadare and J. Cheng, *BMC Bioinf.*, 2017, **18**, 480.
214. W. Shao, J. Wang, Y. Zhang, C. Zhang, J. Chen, Y. Chen, Z. Fei, Z. Ma, X. Sun and C. Jiao, *Nucleic Acids Res.*, 2024, **52**, 4906-4921.
215. T. Liu and Z. Wang, *Bioinformatics*, 2019, **35**, 4222-4228.
216. Z. Li and Z. Dai, *Front. Genet.*, 2020, **11**, 353.
217. D. E. Cook, H. M. Kramer, D. E. Torres, M. F. Seidl and B. Thomma, *Elife*, 2020, **9**, e62208.
218. S. Schwartz, S. D. Agarwala, M. R. Mumbach, M. Jovanovic, P. Mertins, A. Shishkin, Y. Tabach, T. S. Mikkelsen, R. Satija, G. Ruvkun, S. A. Carr, E. S. Lander, G. R. Fink and A. Regev, *Cell*, 2013, **155**, 1409–1421.
219. W. Chen, H. Tran, Z. Liang, H. Lin and L. Zhang, *Sci. Rep.*, 2015, **5**, 13859.
220. X. Qiang, H. Chen, X. Ye, R. Su and L. Wei, *Front. Genet.*, 2018, **9**, 495.
221. N. Q. K. Le and L. Xu, *ACS Omega*, 2023, **8**, 39420–39426.