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REVIEW

Epigenetic modifiers as inducer of bioactive secondary metabolites in fungi

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Abstract Scientists are making efforts to search for new metabolites as they are essential lead molecules for the drug discovery, much required due to the evolution of multi drug resistance and new diseases. Moreover, higher production of known drugs is required because of the ever growing population. Microorganisms offer a vast collection of chemically distinct compounds that exhibit various biological functions. They play a crucial role in safeguarding crops, agriculture, and combating several infectious ailments and cancer. Research on fungi have grabbed a lot of attention after the discovery of penicillin, most of the compounds produced by fungi under normal cultivation conditions are discovered and now rarely new compounds are discovered. Treatment of fungi with the epigenetic modifiers has been becoming very popular since the last few years to boost the discovery of new molecules and enhance the production of already known molecules. Epigenetic literally means above genetics that actually does not alter the genome but alter its expression by altering the state of chromatin from heterochromatin to euchromatin. Chromatin in heterochromatin state usually doesn't express because it is closely packed by histones in this state. Epigenetic modifiers loosen the packing of chromatin by inhibiting DNA methylation and histone deacetylation and thus permit the expression of genes that usually remain dormant. This study delves into the possibility of utilizing epigenetic modifying agents to generate pharmacologically significant secondary metabolites from fungi.

Keywords DNMT inhibitors · Epigenetic modifiers · Fungi · HDAC inhibitors · Natural products · Secondary metabolites

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Introduction

The rise of bacterial resistance to existing antibiotics is a major cause for concern and highlights the urgent need for the discovery of novel antibiotics to meet the increasing demands of modern medicine. Alternative strategies are needed to boost the production of new chemical compounds and avoid rediscovery of natural products (Scherlach and Hertweck 2009).

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Fungi are excellent source of food and medicines and 42 per cent of over 20,000 bioactive compounds are produced by fungi. Recent studies have indicated that numerous genes in fungi remain inactive, and the utilization of epigenetic modifying agents is a crucial method to enhance the chemical variety of secondary metabolites produced in laboratory settings by fungi. (Ibrahim et al. 2023; Kamat et al. 2023; Tiwari and Bae 2022). The potential of using epigenetic modifiers to induce changes in fungal secondary metabolism have recently come to light (Makhwitine et al., 2023). Epigenetic modifiers offer their use at desired concentration by using dose dilution. Their use require optimization of their concentration as higher doses of epigenetic modifiers are inhibitory to the cultures (Vasanthakumari et al. 2015). The effect of treatment of fungi with epigenetic modifiers can be gauged by using HPLC, MS and TLC. In one study, eleven of twelve fungi either increased production of already known compounds or started production of new metabolites on treatment with epigenetic modifiers (Williams et al. 2008). Endophytic fungal cultures respond modestly to the treatment of both the HDAC and DNMT inhibitors and their combined effects are not additive. The cultures that respond to the epigenetic treatment do not respond to amphotericin B, cycloheximide, and 5-fluorouracil (Williams et al. 2008). Epigenetic modifying agents can be broadly classified into two categories—Histone deacetylase inhibitors (HDACi) and DNA methyl transferase inhibitors (DNMTi) (Xue et al. 2023; Bind et al. 2022; Gupta et al. 2020). The DNMTi include 5-aza-2′-deoxycytidine, hydralazine, procaine, 5-azacytidine and procainamide whereas the HDACi include suberoylanilide hydroxamic acid (SAHA), sodium butyrate, suberohydroxamic acid/ suberic*bis*- hydroxamic acid (SBHA), valproic acid and trichostatin A. Moreover, some protease inhibitors also proved to be useful in the synthesis of new compounds (VanderMolen et al. 2014). In this study, role of epigenetic modifiers and Proteasome inhibitors in epigenetic modulation is discussed. We have suggested the use of epigenetic modifiers for screening of endophytes for the new secondary metabolites with therapeutic applications. We have also discussed the therapeutic use of epigenetic modifiers and the compounds produced by the fungi on treatment with the epigenetic modifiers. Employing epigenetic modifying agents may enhance our capability to screen and isolate therapeutic compounds from endophytic fungi.

Why epigenetic modifiers for the induction of natural products

Genomic investigations of endophytic fungi have uncovered their immense potential for natural product synthesis, which has yet to be fully harnessed due to the excessive reliance on traditional methods for discovering new molecules. Endophytes exhibit a higher potential for producing compounds compared to those discovered through classical bioactivity screening. The number of genes encoding biosynthetic enzymes involved in secondary metabolite production is considerably greater than the number of metabolites produced by various fungi and bacteria. Microbial production of identical compounds is attributed to the expression of the same set of genes under normal conditions. To realize the potential of unexplored cryptic or orphan pathways for the synthesis of unique compounds with novel structures, activation of silent genetic loci is necessary (Chung et al. 2013; Scherlach and Hertweck 2009). Researchers are presently focusing on inhibiting Histone deacetylase (HDAC) and DNA methyl transferase (DNMT) as it leads to the activation of silent biosynthetic genes (Xue et al. 2023; Verma et al. 2023). Epigenetic modifiers are small molecules that facilitate the production of novel secondary metabolite structures in fungi (Fig. 1).

Methylation of cytosine in DNA by DNA methyltransferase suppresses transcription and switches off genes. The insertion of acetyl groups to histones of nucleosome by histone acetylase starts transcription and switches on gene. HDACi and DNMTi are known epigenetic modulators of secondary metabolites in fungi and many examples illustrating the potential of epigenetic modifiers in induction of secondary metabolite biosynthesis in fungi are discussed here (Fig. 2; Tables 1 and 2).

DNA methyl transferase inhibitors role in humans and in epigenetic modulation of fungi

DNA methylation is an epigenetic procedure of chromatin renovation by DNA methyl transferases that control gene expression. DNA methyltransferases

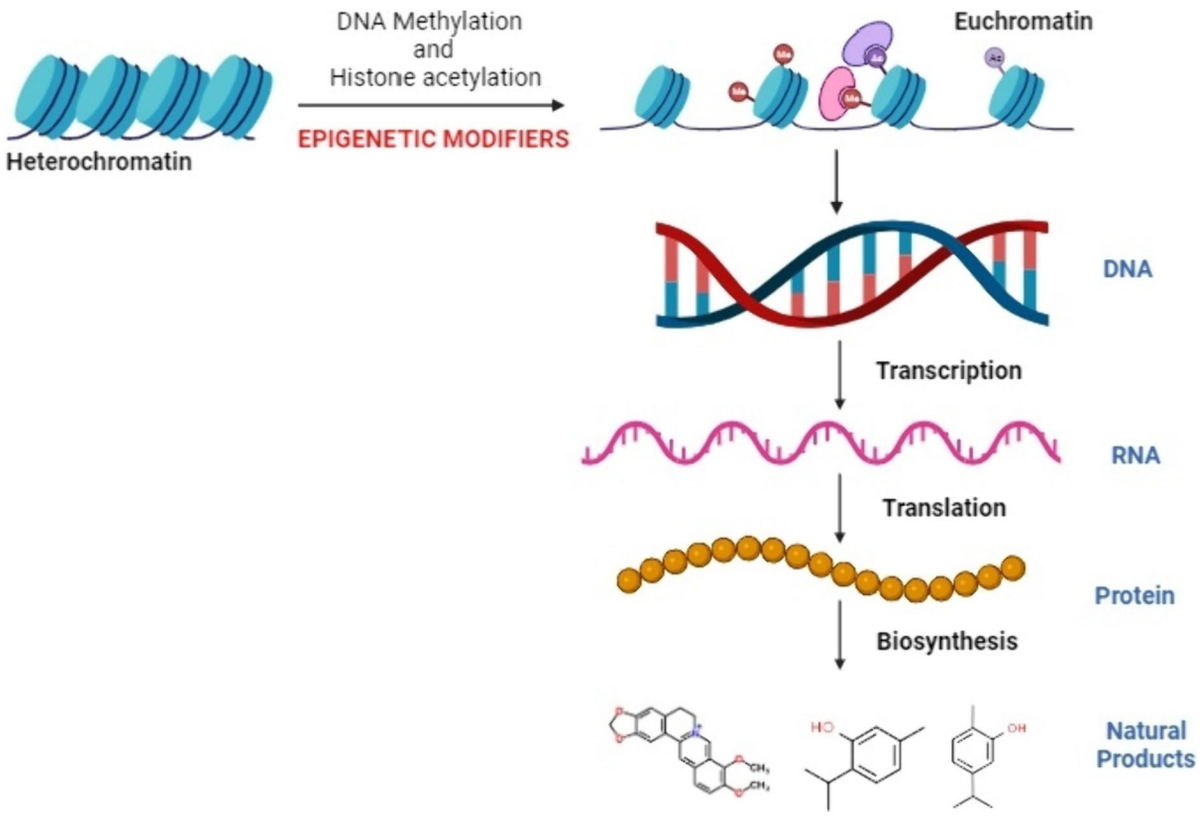


Fig. 1 Outline illustrating the impact of epigenetic modifiers on genetic information flow in fungi/eukaryotes leading to biosynthesis of secondary metabolite

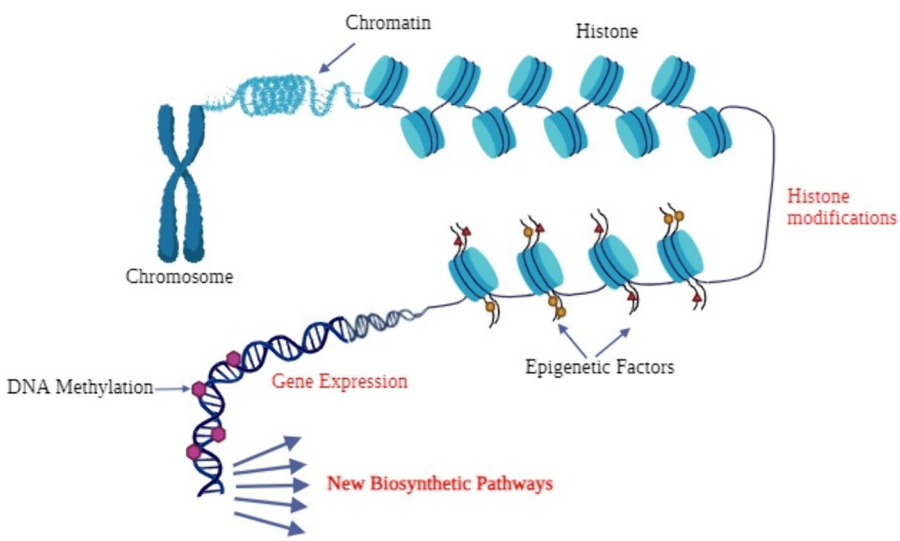


Fig. 2 Outline illustrating epigenetic modifiers leading to secondary metabolite biosynthesis in fungi

Table 1 Compounds isolated from fungi on treatment with DNA methyltransferase inhibitors (DNMTi)

Taxonomic designation of fungi	Source of fungi	DNMT inhibitor used	New secondary metabolite produced	References
Phomopsis asparagi	Fresh root of the Rhizophora mangrove plant mangle, collected from Dong Zhai Gang-Mangrove Garden located on Hainan Island, China	DNA methyltransferase (DNMT) inhibitor 5-azacytidine (5-Aza)	phomoparagin D (**5**) andphaseolorin J (**1**)	Feng et al. (2022)
Attenuated *Botryosphaeria rhodina*	Camptothecine producing *Nothapodytesnimmoniana*	5-azacytidine	Enhanced camptothecine content	Vasanthakumari et al. (2015)
Aspergillus sydowii	Marine sediment, Hsinchu, Taiwan	5-azacytidine	3 new bisabolane-type sesquiterpenoids along with eight known compounds	Chung et al. (2013)
Cordyceps indigotica	Entomopathogenic fungus	5- azacytidine	A new aromatic polyketide glycoside, indigotide B	Asai et al. (2012c)
Penicillium citreonigrum(syn. Eupenicillium hirayamae)	Atlantic forest soil	5-azacytidine	Two new meroterpenesi.eatlantinones A and B and six azaphilones	Wang et al. (2010)
Cladosporium cladosporioides	Sample of sediment collected from a tidal pool located along the coastline of Casco Bay in Portland, Maine, USA	5-azacytidine	Oxylipins such as (9Z,12Z)-11-hydroxyoctadeca-9,12-dienoic acid, its methyl ester, and glycerol conjugate	Williams et al. (2008)
Diatrype disciformis	Foregut of a moth larva	5-azacytidine	New polyketides, Lunalides A and B	Williams et al. (2008)
Neurospora crassa		5-azacytidine	Increased production of carotenoids	Kritsky et al. (2001)

Table 2 Compounds isolated from fungi on treatment with histone deacetylase inhibitors (HDACi)

Taxonomic designation of fungi	Source of fungi	Epigenetic modifier used	New secondary metabolite produced	References
Aspergillus calidoustus and *Aspergillus westerdijkiae*	Not specified	Vorinostat	Modulation of secondary metabolite profiles	Xue et al. (2023)
Aspergillus terreus	*A.terreus* RA2905 isolated from the sea hare	Histone deacetylase HdaA	azaphilone derivatives. Two new azaphilones, asperterilones A and B (1 and 2)	Zheng et al. (2022)
Nigrospora sphaerica	Endophytic fungi	Sodium butyrate, subero hydroxamic acid (SAHA), and valproic acid	Cryptic metabolites	Ramesha et al. (2021)
Botryosphaeria mamane	*Bixaorellana*	SAHA	Eight metabolites	Triastuti et al. (2019)
Botryosphaeria mamane	*Bixaorellana*	Sodium valproate	Two metabolites	Triastuti et al. (2019)
Diaporthe sp.	*Datura innoxia* Mill	Valproic acid	Three new compounds diportharine A, xylarolide A, and xylarolide B	Sharma et al. (2018)
Aspergillus fumigatus	*Grewia asiatica*	Valproic acid	Increase in yield of fumiquinazoline C	Magotra et al. (2017)
Aspergillus nidulans		HDACi	15 new aspercryptins	Henke et al. (2016)
Daldinia sp.	*Chrysophyllum cainito*	Suberoylanilidehydroxamic acid	Daldinone E	Du et al. (2014)
Fusarium oxysporum	Roots of plant *Datura stramonium*	Suberoylanilide hydroxamic acid	Fusaric acid derivatives- 5-butyl-6-oxo-1,6-dihydropyridine-2-carboxylic acid and5-(but-9-enyl)-6-oxo-1,6-dihydropyridine-2-carboxylic acid	Chen et al. (2013)
Gibellula formosana		YM medium supplemented with both RG-108 (1 mM) and SBHA (1 mM)	Five new isariotin analogs, two new highly oxidized ergosterols-formosterols A and B	Asai et al. (2012a)
Isaria tenuipes	Entomopathogenic fungus	Concomitant addition of RG-108 (500 µM) and SBHA (500 µM)	Tenuipyrone, novel polyketide and cephalosporolides B and F	Asai et al. (2012b)
Leucostoma persoonii	Marine fungus	Sodium butyrate (100 µM), 7 days	Enhanced production of a new cytosporone R. and known cytosporones B, C and E	Beau et al. (2012)
Torrubiella luteorostrata	Entomopathogenic fungus	SBHA, 1 mM SBHA, 18 days	Three novel prenylated tryptophan analogs- luteoride A, B and C along with terezine D and paecilodepsipeptide A	Asai et al. (2011)
Aspergillus niger		Suberoylanilide hydroxamic acid	Novel compound nygerone A	Henrikson et al. (2009)

Table 2 (continued)

Taxonomic designation of fungi	Source of fungi	Epigenetic modifier used	New secondary metabolite produced	References
Cladosporium cladosporioides	Sediment sample obtained from a tidal pool located along the coastline of Casco Bay in the United States	Suberoylanilide hydroxamic acid	A complex series of perylenequinones, including two new metabolites named cladochromes F and G, as well as four already known metabolites (cladochromes A, B, D, and E) and calphostin B	Williams et al. (2008)
Alternaria alternate		Trichostatin A (1 µM)	Increase the concentrations of many unidentified natural products	Shwab et al. (2007)
Penicillium expansum		Trichostatin A (1 µM)	Increase the concentrations of several unidentified natural products	Shwab et al. (2007)

refer to a group of enzymes responsible for transferring a methyl group onto DNA. The DNA methylation pattern plays a significant role in controlling distinct genome tasks. DNA methylation is a reversible process like other physiological biochemical changes. The DNMT inhibitors decitabine and azacytidine are the most powerful epigenetic modifiers till date and are still the most extensively utilized among other modifiers but their use against cancer is confined due to their relative poisonousness and chemical instability (Patnaik et al. 2023; Hu et al. 2021). Some molecules similar to nucleoside having modified cytosine act as DNMT inhibitors. DNMTidecitabine or azacitidine are analogs of 2′-deoxycytidine and cytidine respectively (Bouyahya et al. 2022).

DNMT inhibitors (DNMTi) have a crucial role in the generation of novel secondary metabolites from endophytic fungi. For example, Entamopathogenic fungi *C. indigotica* produces a new metabolite, indigotide B, on repeated cultivation under 5-azacytidine, a DNMTi. Repeated culturing under 5-azacytidine can give rise to new metabolites because it decreases the number of methylated cytosine in the CpG islands (Asai et al. 2012c). Two new meroterpenes i.e. atlantinones A and B and six azaphilones were produced by *Penicillium citreonigrum* when grown on a solid-state medium based on vermiculite containing 50 µM 5-azacytidine for 20 days (Wang et al. 2010). In one of the studies, *Pestalotiopsis crassiuscula,* anendophytic fungi grown in culture media containing 5-azacytidine have shown change in metabolites and resulted in the biosynthesis of a novel compound coumarin (Yang et al. 2014).The addition of 5-azacytidine to the culture broth of *Aspergillus sydowii* grown on a vermiculite-based solid-state medium led to the synthesis of three new bisabolane-type sesquiterpenoids (7S, 11S)-(+)-12-hydroxysydonic acid, 7-deoxy-7,14-didehydrosydonol and 7S)-(+)-7-O-methylsydonol, and) and eight known compounds. The culture was prepared by dissolving PDB in 1 L of seawater treated with 100 µM of 5-azacytidine and grown on a rotatory shaker (150 rev min^{-1}) at 25 °C for 10 days. Similarly, the addition of low doses of 5-azacytidine ($\leq$30 µM) to N. crassa cultures resulted in increased production of carotenoids. (Kritsky et al. 2001). A recent study investigated the effect of DNMT inhibitors on the treatment of solid tumors and the potential of combining DNMT inhibitors with chemotherapeutic drugs for epigenetic therapy (Hu et al. 2021).

The 5-AZA and Decitabine [5-aza-2′-deoxycytidine] (DNMTi) are mostly used in studies to understand how DNA methylation influence the physiology of endophytes. These man-made drugs are similar to cytidine but they contain a nitrogen atom at 5th position of the pyrimidine ring instead of carbon. Consequently, they become part of the DNA and stops the DNMT from suitably shifting the methyl group. Successive DNA replication cycles results into submissive demethylation. DNMTs remain attached to the DNA even in the presence of DNMTi, but the proteasome pathway breaks them down later on (Santi et al. 1984). The ribonucleoside analogue 5-AZA is present in the RNA molecule as well as in the DNA molecule in limited quantity but the deoxyribose analogue decitabine is present only in DNA (Gnyszka et al. 2013).

HDAC inhibitors role in humans and in epigenetic modulation of fungi

HDAC inhibitors are compounds that have the ability to enhance the acetylation of lysine amino acids in both histone and non-histone proteins by inhibiting the activity of HDAC enzymes. These enzymes usually remove acetyl groups from the lysine amino acids in the amino terminal tails of core non-histone and histone proteins, which leads to the formation of a tightly packed chromatin structure, making it difficult for transcription factors to bind to DNA, resulting in the suppression of expression of gene. Depending on their location in the cell, HDAC enzymes are classified into three classes. For example, Class I HDAC enzymes include HDAC 1, 2, 3, and 8, which are mainly localized in the nucleus (Li et al. 2020). Humans have 18 HDAC enzymes that use either NAD + or Zn dependent pathways for deacetylation of acetylated lysine residues in non-histone and histone proteins. HDACs are responsible for the removal of the acetyl group from histones, which can influence gene expression and other physiological processes in humans and fungi (Bassett and Barnett, 2014; Li et al. 2020). HDAC inhibitors have potential applications in cancer therapy, as they can induce transcriptional reprogramming and affect the expression of genes involved in transcriptional regulation, metabolism, angiogenesis, DNA damage response, cell cycle, apoptosis, protein degradation, immunity, and other physiological processes (Li et al. 2020; Verza et al. 2020). HDAC inhibitors are effective anti-cancer remedies, especially when used in combination with radiotherapy and/or other anti-cancer drugs. Romidepsin, vorinostat, belinostat, and panobinostat are commonly used HDAC inhibitors for the treatment of multiple myeloma and some T-cell lymphoma (Cappellacci et al. 2020). HDAC inhibitors are compounds that can increase the expression of the cell cycle gene p21 in various cancer cell lines. This increase leads to the arrest of the cyclin/CDK complexes, which then stops the cell cycle and prevents differentiation. Additionally, HDAC inhibitors can regulate the balance between anti- and pro-apoptotic proteins, which causes cancer cells to die. In humans, sodium valproate, an antiepileptic drug, is a non-specific inhibitor of HDAC9 (Brookes et al. 2018).

Histones acetylation is known to increase gene expression through activation of transcription. Inhibition of HDAC enzymes results into hyper acetylation of core histones of nucleosome in most parts but activates only few genes and deactivates large or equal number of other genes. The addition of HDAC inhibitors to various fungal cultures has resulted in the production of novel compounds. For example, *Aspergillus niger* produced nygerone A when treated with suberoylanilide hydroxamic acid, while *Cladosporium cladosporioides* produced perylenequinones and new metabolites i.e., cladochromes F and G, claphostin B and four known cladochromes A,B,D and E when treated with the same HDAC inhibitor (Henrikson et al. 2009; Williams et al. 2008). An entomopathogenic fungus, *Isariatenuipes*, produced tenuipyrone, cephalosporolides B and F and new polyketide when treated with both SBHA and RG-108 (Asai et al. 2012b). Similarly, *Torrubiella luteorostrata* produced novel prenylated tryptophan analogs and paecilodepsipeptide A when treated with SBHA (Asai et al. 2011). *Gibellula formosana* produced highly oxidized ergosterols and isariotin analogs when treated with both RG-108 and SBHA (Asai et al. 2012a). *Fusarium oxysporum* produced fusaric acid derivatives when treated with suberoylanilide hydroxamic acid (Chen et al. 2013), and increased concentrations of unknown natural products were detected in cultures of *Alternaria alternata* and *Penicillium expansum* when treated with trichostatin A (Shwab et al. 2007). *Leucostoma persoonii* produced

known cytosporones B, C, and E, and a new cytosporone R when treated with sodium butyrate (Beau et al. 2012). *Aspergillus nidulans* produced 15 new aspercryptins when treated with an HDAC inhibitor (Henke et al. 2016). In *Botryosphaeria mamane*, the addition of SAHA and sodium valproate resulted in the upregulation and downregulation of metabolites, and the detection of 12 compounds structurally related to SAHA in the medium, while the cultivation of B. mamane in the presence of SAHA and sodium valproate yielded eight and two metabolites, respectively, that were not detected in the strain cultivated without the inhibitors (Triastuti et al. 2019).In one study, treatment of *Nigrospora sphaerica* withHDACi significantly increased induction of cryptic secondary metabolites than treatment with DNMTi. Treatment of *Nigrospora sphaerica* with sodium butyrate, SAHA, and Valproic acid resulted in the induction of 22, 19 and 10 cryptic secondary metabolites respectively (Rameshaet al. 2021).

Reversion of attenuation of fungi by epigenetic modifiers

Endophytes have the potential to produce various compounds that can be useful in medicine, agriculture, and industry (Strobel and Daisy 2003). These compounds are synthesized by the host plants of the endophytes, making them an alternative source of plant secondary metabolites that can reduce our dependence on the host plants themselves (Li et al. 2023; Shweta et al. 2013; Stierle et al.1993). However, despite these expectations, endophytes often produce low quantities of metabolites and can lose their ability to produce them when sub-cultured, a phenomenon known as attenuation (El-Elimat et al. 2014; Kusari et al. 2011; Li et al. 1998). The exact mechanism behind attenuation is not yet fully understood, but it may be due to gene silencing during successive sub-culturing or the absence of host signaling in axenic cultures (Kumara et al. 2014; Priti et al. 2009). Many efforts like culturing the endophyte with the extracts of tissues of the host plant were done to revive the attenuated cultures but were failed (Gurudatt et al. 2010; Kusari et al. 2011). Endophytic fungus *Botryosphaeria rhodiana* isolated from *Nothapodytes nimmoniana* produced camptothecine but lost its capability to synthesize camptothecine on sub-culturing. Utilization of 5- azacytidine, a DNMTi that blocks DNA methyl transferase leads to the reversal of the ability of the culture to produce camptothecine. *Phomopsis sp.*, an endophyte isolated from camptothecine producing plant *Miqueliadentata,* also lost its ability to produce camptothecine by sixth subculturing but regained its ability to produce camptothecine when passed by a non-host plants in-vitro that produces camptothecine such as *Nothapodytes nimmoniana* and *Ophiorrhiza mungos.* Thissuggests that the production of camptothecine by plants is linked to certain signaling mechanisms that help maintain the fungus's ability to produce the compound endogenously. Without these signals, the genes responsible for producing camptothecine in the fungus may become silent. Fungi become attenuated on several sub-culturing and lose its ability to produce camptothecine but use of 5- azacytidine partially reversed the attenuation of fungi. This suggests that signalling that occurs when endophyte is in its host prevents its DNA from DNA methylation and thus maintains the expression of genes responsible for producing secondary metabolites. Sub-culturing in lab conditions leads to DNA methylation and thus silencing of many genes that express in nature. 5-azacytidine added to the cultures growing in fermenters also perform a function similar to the host plant by preventing DNA methylation and thus leads to gene expression and secondary metabolite biosynthesis. Thus artificial stimulus to the silent genes can be provided by 5-azacytidine. 5-azacytidine treatment (1, 5, 7.5, 10 µM) after incubating *Botryosphaeria rhodina* for 3 days at 25 °C on a rotary shaker at 170 rev min^{-1} and then growing for an additional 5 days can reverse the attenuation. Reversal of attenuation in *Botryosphaeria rhodina* can also be achieved either by inoculation and re- isolation of the attenuated fungi from the plant that produces campothecine or by addition of 5-azacytidine in-vitro. Re-isolated *Botryosphaeria rhodina* from the site of the inoculation of its attenuated strain was a better producer of the camptothecine than the *Botryosphaeria rhodina* isolated away from site of inoculation of attenuated *Botryosphaeria rhodina*(Vasanthakumari et al. 2015).

Proteasome inhibitors in epigenetic modulation

Proteasomes are the protein complexes that degrade proteins by proteolysis and some of the proteins degraded by proteasome have been identified as transcriptional regulators which play significant role in gene transcription (Anjum and Xuewei 2022; Toghueo et al. 2020; Zimmermann et al. 2000). The proteasome is a network of lot of proteins that break down substrates marked by ubiquitin. Ubiquitin–proteasome-mediated proteolysis has a role in memory and synaptic plasticity. Proteasome clear unwanted histone proteins and thus play an important role in epigenetic regulation of transcription of target genes. Proteasome play proteolytic and non-proteolytic roles in post translation alhistone modifications like methylation, ubiquitination and acetylation that affect memory and synaptic plasticity (Bach and Hegde 2016). Proteasome inhibition can increase transcription output probably due to increased levels of trimethyl H3K4 and phosphorylated RNA polymerase (Pol) II at the gene body and promoter (Kinyamu et al. 2020).In one study, use of bortezomib, a proteasome inhibitor on a filamentous fungus, *Mycosynthetix fungal* strain 63935 (order *Pleosporales*), has been proved beneficial for discovering a new compound and identifying a previously reported compound from a plant whose structure was unknown. *Mycosynthetix fungal* strain 63935 was cultivated in 250 ml Erlenmeyer flasks containing 50 ml of PDB and 0.5 ml of bortezomib dissolved in DMSO (a proteasome inhibitor) was added in triplicate to 15 flasks. The final concentration of bortezomib in the flask was 75 mg ml^{-1} at the time of inoculation and were kept at 100 rev min^{-1} and 22 °C for 14 days (VanderMolen et al. 2014). Proteasome inhibitor was used for the first time for epigenetic modulation and this might be an inspiration for a new strategy to up-regulate the silent genes and it came into light that secondary metabolites produced by fungus under the influence of bortezomib were similar as in case of 5-azacytidine thus it can act as an alternative tool for genome mining (Table 3).Thousands of mysterious secondary metabolism biosynthesis gene clusters that give rise to precious compounds are revealed by fungal genome projects. For instance, biosynthetic pathway of a proteasome inhibitor i.e., fellutamide B in *Aspergillus nidulans*was revealed by serial promoter exchanges (Yeh et al. 2016).

Biological importance of compounds produced by fungi on addition of epigenetic modifiers

A number of small molecules produced by epigenetic modulation of fungi have great biological significance and shows antimicrobial, antioxidant, anticancer and apoptotic activities. Some of the compounds having biological significance are discussed below:

Compounds having antimicrobial activity

Cytosporones (Fig. 3) produced by *Leucostoma persoonii on treatment with* sodium butyrate (100 µM) have antimicrobial activity against *Salmonella enterica serovar Typhimurium* and *Plasmodium falciparum.* Cytosporone B analogs effectively block the secretion of proteins associated with Salmonella pathogenicity island 1 (SPI-1) in vitro. In contrast, Cytosporone E exhibits moderate activity against Plasmodium falciparum and the highly resistant USA100 strain of MRSA, with low cytotoxicity towards mammalian cells, providing a therapeutic index greater than 10 for both infectious diseases

Table 3 Compound isolated from fungi on treatment with Protease inhibitor

Taxonomic designation of fungi	Source of fungi	Epigenetic modifier used	New secondary metabolite produced	References
Penicillium chrysogenum	Endophytic fungi	Bortezomib	Additional secondary metabolite	Xue et al. (2023)
Aspergillus sp. SCSIOW3	Marine-derived fungi	Epigenetic modifying agents	Enhanced Secondary metabolites	Xue et al. (2023)
Myco synthetix fungal strain 63935(order *Pleosporales*)	Leaf litter	DMSO-dissolved Bortezomib, a proteasome inhibitor	Isolation of a new compound and a known compound	VanderMolen et al. (2014)

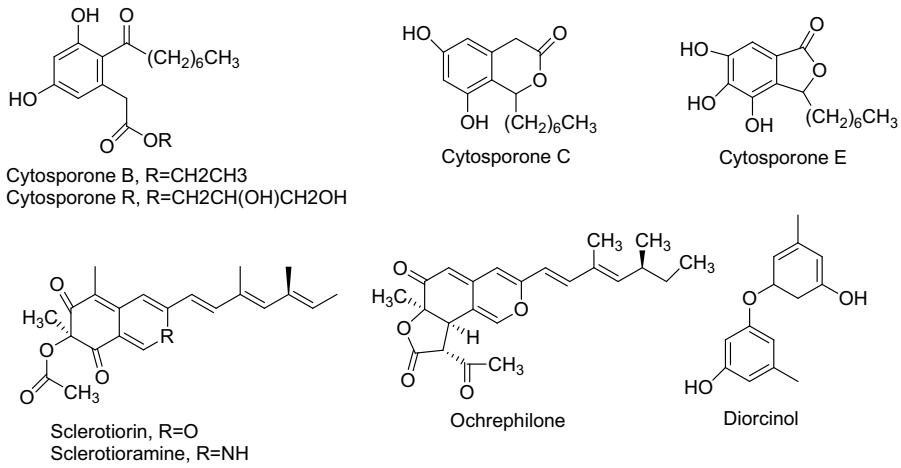


Fig. 3 Structures of the antimicrobial compounds isolated after treatment with epigenetic modifiers

(Beau et al. 2012; Li et al. 2013). Two of the six azaphilones viz. sclerotiorin and sclerotioramine isolated from *Penicillium citreonigrum* by using 5-azacytidine have moderate activity against *Staphylococcus epidermidis* while a third azaphilone-ochrephilone is an inhibitor of gp120-CD4 and is a binding agent of endothelin receptors (Yang et al. 2006; Wang et al. 2010). Moreover, sclerotioramine is active against a panel of candida strains (Wang et al., 2010). Diocinol D is obtained from *Aspergillus sydowii*, a type of fungus that grows inside lichens, by using a chemical called 5-azacytidine. Diocinol D is a type of chemical that is related to diphenyl ether. It has the ability to kill the fungus *Candida albicans* by causing an accumulation of reactive oxygen species and damaging its cell membrane. (Chung et al. 2013; Li et al. 2015).

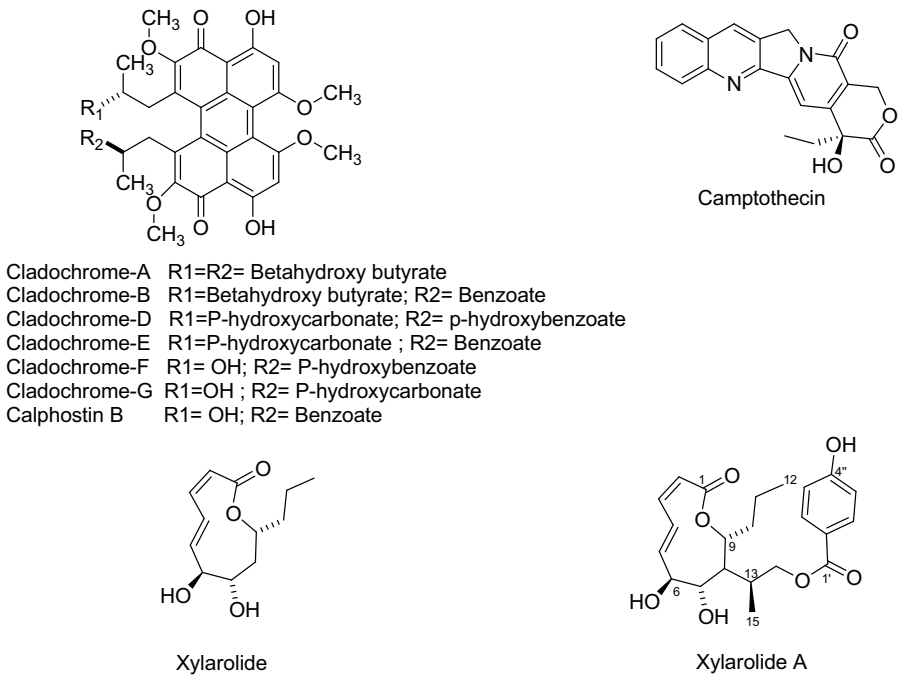


Fig. 4 Structures of anticancer and apoptotic compounds extracted from fungi on treatment with epigenetic modifiers

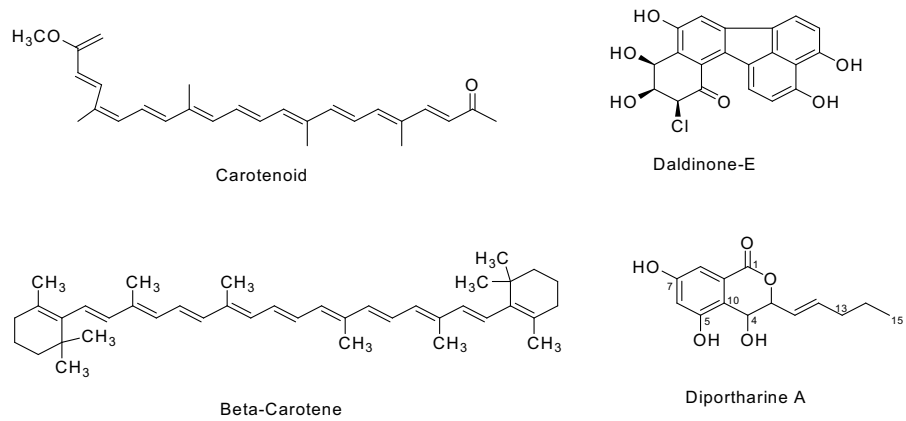


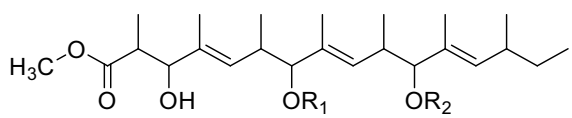
Fig. 5 Structures of antioxidant compounds extracted from fungi after treatment with epigenetic modifiers

Compounds having anticancer and apoptotic activities

A complex series of perylenequinones including four known metabolites cladochrome A, B, D, E, two new metabolites cladochromes F and G, and calphostin B were reported from *Cladosporium cladosporioides* after addition of suberoylanilide hydroxamic acid (Fig. 4). These compounds possess the ability to elicit apoptotic activity by specifically binding to the C1 regulatory domain of protein kinase C. This binding event initiates a complex signaling cascade, ultimately leading to the activation of downstream apoptotic effectors such as caspases. Through this mechanism, the compounds are able to induce programmed cell death, or apoptosis (Mulrooey et al. 2012; Williams et al. 2008). 5-azacytidine have the ability to revive the attenuated *Botryosphaeria rhodina* isolated from camptothecine producing *Nothapodytes nimmoniana* plant to produce higher yield of quinoline alkaloid camptothecin which is cytotoxic and inhibits the enzyme DNA topoisomerase I (Vasanthakumari et al. 2015). Xylarolide A and Xylarolide are chemical compounds that have been extracted from *Diaporthe* sp. The compounds exhibited significant antioxidant activity and demonstrated an ability to inhibit the growth of pancreatic cancer cell line MIAPaCa-2 and prostate cancer cell line PC-3. This inhibition was observed when the *Diaporthe* sp. was grown in the presence of valproic acid (Sharma et al. 2018).

Compounds having antioxidant activity

Production of antioxidant compounds like Carotenoids, Beta-carotene and Daldinone-E has been reported from various fungal cultures on growing them in presence of epigenetic modifiers (Fig. 5). Increased production of carotenoids have been reported from *Neurospora crassa* after addition of 5-azacytidine. Carotenoids protect us from the damage done by oxygen and light. Carotenoids possess antioxidant properties and are capable of quenching singlet oxygen as well as trapping peroxyl radicals. This ability makes carotenoids an important contributor to the maintenance of cellular homeostasis by protecting cells from oxidative damage caused by free radicals and other reactive oxygen species. Carotenids are useful as animals including humans don't synthesize them and have to rely upon their diet from other sources. Carotenoids protect animals from skin burns and act as immune system enhancer. Beta carotene, a carotenoid acts as a precursor of vitamin A (Kritsky et al. 2001; Mathews-Roth 1990; Stahl and Sies 1996; Van den Berg et al. 2000). Chlorinated polyketide Daldinone-E isolated from *Chrysophyllum cainito* after treatment with suberoylanilide hydroxamic acid has been shown to exhibit a significant capacity for scavenging DPPH radicals, with IC50 values of 3.6 µM comparable to that of ascorbic acid, a well-known antioxidant, which has an IC50 value of 3.2 µM (Du et al. 2014). Diaportharine A



Lunalide A R1=H; R2=Beta-D-mannopyranoside
Lunalide B R1= R2=Beta-D-mannopyranoside

Fig. 6 Structures of the Lunalide A and B isolated from fungi after treatment with epigenetic modifiers

and Xylarolide A isolated from *Diaporthe* sp. showed significant antioxidant activity having EC_{50} value of 10.3 and of 27.5 µM respectively when cultivated in presence of valproic acid (Sharma et al. 2018).

Compounds having multiple bioactivities

Lunalide A and B and new polyketides, were extracted from *Diatrype disciformis* on treatment with 5-azacytidine (Fig. 6). They are known to have antimicrobial, antifungal, anti-parasitic, antitumor and agrochemical properties (Williams et al. 2008).

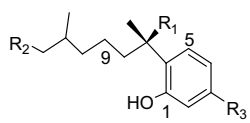
Compounds having miscellaneous activities

Compounds having miscellaneous activities like sydonols, sydonoic acid and oxylipins are shown in Fig. 7. 5-azacytidine treatment of *Aspergillus sydowii* results in the production of (S)-(+)-sydonol.(S)-(+)-sydonol increases glucose consumption stimulated by insulin and prevents lipid growth in 3T3-L1 adipocytes. (S)-(+)-Sydonol exhibits notable anti-inflammatory activity by inhibiting the generation of superoxide anions and the release of elastase by fMLP/CB-induced human neutrophils. (S)-(+)-Sydonol is the first secondary metabolite derived from microorganisms to demonstrate both anti-inflammatory activities and anti-diabetic (Chung et al. 2013). Oxylipins reported from *Cladosporium cladosporioides* on treatment with 5-azacytidine are involved in intra and inter-species cell signaling (Williams et al. 2008). Compounds isolated from *Aspergillus sydowii* after treatment with 5-azacytidine have anti-inflammatory and anti-diabetic activity with (S)-(+)-sydonol (Chung et al. 2013; Li et al. 2015).

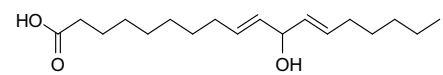
Varied classes of compounds produced by endophytes on treatment with epigenetic modifiers

Microbes are an important source of natural products used in the drug discovery and development.

Several secondary metabolites obtained from microorganisms or their derivatives are used as drugs in the treatment of fatal diseases such as cyclosporine, lovastatin and penicillin. Treatment of fungi with epigenetic modifiers is practically used by researchers for the production of heterocyclic ring structures and novel secondary metabolites with new structures (Li et al. 2020). Some species of endophytic fungi produce varied classes of secondary metabolites when grown under the influence of epigenetic modifiers. The classes include complex perylenequinones, cytosporones, azaphilones, quinolone, alkaloids, oxylipins, polyketides, carotenoids, bisabolane-type sesquiterpenoids, xanthones analogs and chlorinated polyketide. The compounds of these classes have great biological significance. The basic biological importance and advanced studies carried on these molecules is shown in Table 4. Perylenequinones class of natural products have pentacyclic conjugated chromophore that give rise to photo activity. Perylenequinones are studied widely because of their light-induced biological activity and structural complexity (Mulrooey et al. 2012). Cytosporones are fungal secondary metabolites that have antimicrobial activity and inhibits proliferation of cancer cell (Brady 2000). Derivatives of cytosporone isolated from an enpdophytic fungi *Phomopsis* sp. act as anti-inflammatory compound by obstructing MAPK/NF-κB signaling route and thus can be developed as anti-inflammatory drugs (Wang et al. 2023). Azaphilones shows extensive biological effects Viz. antifungal, antibacterial, antiviral, cytotoxic, antioxidant, anti-inflammatory and nematicidal effects. Many of these



(7S)-(+)-7-O-methylsydonol, R1=OMe, R2=H, R3=CH2OH
(S)-(+)-sydonol, R1=OH, R2=H, R3=CH2OH
(S)-(+)-sydonic acid,R1=OH, R2=H, R3=COOH



(9Z,12Z)-11-hydroxyoctadeca-9,12-dienoic acid

(Oxylipin)

Fig. 7 Structures of compounds with miscellaneous bioactivities, isolated after treatment with epigenetic modifiers

Table 4 Compounds of varied classes synthesized by endophytic fungi grown in presence of epigenetic modifiers and their biological significance

Class of molecules obtained	Name of Secondary metabolite	Biological importance	References
Coumestans	Coumestan-type	Anti-HIV-1 activity against integrase	Makhwitine et al. (2023)
Siloxanes	Cyclotrisiloxane, hexamethyl; Cyclotetrasiloxane, octamethyl; Cyclopentasiloxane, decamethyl	Stronger anti-HIV-1 properties	Makhwitine et al. (2023)
Alkaloids	Lepiotaprocerine G	Potential blocker of SARS-CoV-2 main protease	Pillay et al. (2022)
Peptides	Cyclosporin A	Immunosuppressive agent	Alam et al. (2021)
Chlorinated polyketide	Daldinone-E	Scavenging DPPH radicals, with IC50 values of 3.6 µM comparable to that of ascorbic acid which has an IC50 value of 3.2 µM	Du et al. (2014)
Cytosporones	A new cytosporone R and Cytosporones B, C, E	Cytosporone B analogs effectively block the secretion of proteins associated with *Salmonella* pathogenicity island 1 (SPI-1) in vitro. Cytosporone E exhibits moderate activity against *Plasmodium falciparum* and the highly resistant USA100 strain of MRSA, with low cytotoxicity towards mammalian cells, providing a therapeutic index greater than 10 for both infectious diseases	Beau et al. (2012); Li et al. (2013)
Perylenequinones	Four known cladochromes A, B, D, and E, two new metabolites cladochromes F and G and calphostin B	Used in Chines herbal medicine and cause apoptosis by binding selectively to the C1 regulatory domain of protein kinase C	Mulrooey et al. (2012); Williams et al. (2008)
Azaphilones	Sclerotiorin	Modest inhibition of *Staphylococcus epidermidis*	Wang et al. (2010)
	Ochrephilone	Inhibitor of gp120-CD4; binding agent of endothelin receptors	Yang et al. (2006)
	Sclerotioramine	Modest inhibition of *Staphylococcus epidermidis* and active against a panel of *Candida* strains	
Quinoline alkaloid	Camptothecine	Cytotoxic and inhibits the enzyme DNA topoisomerase I	Vasanthakumari et al. (2015);Wu et al. (2010)
Oxylipins	Glycerol conjugate, (9*Z*,12*Z*)-11-hydroxyoctadeca-9,12-dienoic acid, its methyl ester	Inter and Intra-species cell signaling	Williams et al. (2008)
Polyketides	Lunalide A and B	Antimicrobial, antifungal, anti-parasitic, antitumor and agrochemical properties	Williams et al. (2008)

Table 4 (continued)

Class of molecules obtained	Name of Secondary metabolite	Biological importance	References
Carotenoids		Protect damage done by light and oxygen in fungi and animals including humans. Animals don't synthesize carotenoids and rely upon the diet for carotenoids which act as antioxidants because they have the ability to scavenge free radicals. Carotenoids does enhancement of function of immune system, protection of skin burns. Beta carotene, a carotenoid serves as a precursor of vitamin A	Kritsky et al. (2001); Mathews-Roth (1990); Stahl and Sies (1996); Van den Berg et al. (2000)
Bisabolane-type sesquiterpenoids and xanthones analogs	Diorcinol	The fungicidal action of a substance against *Candida albicans* involves two mechanisms: cytoplasmic membrane destruction and accumulation of reactive oxygen species (ROS)	Chung et al. (2013); Li et al. (2015)
	(7S)-(+)-7-O-methylsydonol, (S)-(+)-sydonol, and (S)-(+)-sydonic acid	Anti-inflammatory and anti-diabetic activity with S)-(+)-sydonol exhibiting the most potent activity	

activitiesare due to the reactions of azaphilones with amino groups of nucleic acids, amino acids and proteins (Osmanova et al. 2010). Compounds based on Quinolone have been reported active against tuberculosis, malaria, helminth and fungal infections etc. (Dube et al. 2023). Hence, the scaffold of perylenequinones, cytosporones, azaphilones, quinolone, alkaloids and other compounds produced by endophytic fungi on treatment with epigenetic modifiers is of considerable interest to many investigators in various subjects.

Major barriers and future directions in the field

Epigenetic modifiers have emerged as a promising avenue for inducing secondary metabolite production in fungi, offering a novel approach to harness the biosynthetic potential of these organisms (Xue et al. 2023). However, several major barriers and future directions need to be addressed to fully unlock the potential of epigenetic modulation in this context.

One major barrier is the limited understanding of the intricate epigenetic mechanisms governing secondary metabolite gene clusters in fungi. Deciphering the epigenetic marks and modifications that control these clusters, such as DNA methylation and histone acetylation, is essential (Keller 2019). Future research should focus on elucidating the specific epigenetic patterns associated with various secondary metabolites, as this knowledge will guide the rational design of epigenetic modifiers to enhance their production.

Secondly, the lack of efficient and specific epigenetic modifiers for fungi is a significant challenge. Current tools for epigenetic modification, such as histone deacetylase inhibitors and DNA methyltransferase inhibitors, may have off-target effects and lack specificity for fungal systems. Developing novel, fungal-specific epigenetic modifiers that can be precisely targeted to relevant gene clusters is an exciting avenue for future research.

Moreover, the environmental and nutritional factors influencing epigenetic regulation in fungi remain poorly understood. Understanding how external conditions impact the epigenetic landscape and, subsequently, secondary metabolite production is crucial. Investigating the interplay between nutrient availability, temperature, and other factors with epigenetic

modifiers will provide valuable insights for optimizing secondary metabolite induction.

Conclusions

The new secondary metabolites are required for the drug development and combating various communicable and non-communicable diseases. This review focuses on recently isolated new compounds and higher production of already known compounds from the fungi on application of epigenetic modifiers. Fungi are a rich source of diverse biologically active compounds that have potential for various medical applications. However, not all of the genes present in fungal genomes are expressed under standard laboratory conditions. To fully realize the potential of fungal-derived compounds, large-scale screening of fungal cultures using epigenetic modifiers is necessary. These modifiers can alter the way that genes are expressed in fungi, allowing for the identification of novel and useful compounds that may not have been previously detected. This approach has the potential to greatly expand the scope of natural products that can be derived from fungi and contribute to the discovery of new drugs and other biologically active compounds.

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Author contributions VS: Writing original draft, Formal analysis. SP: Draft manuscript preparation, Editing and Review. NS: Conceived and designed the analysis. VA: Drafted the article and revised. UG: Formal analysis and review. SJ: Conceptualization, Formal analysis, Final review, Editing and supervision.

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Declarations

Competing interest The authors declare that they have no conflict of interests.

Ethical approval There is no study in this article that involves the participation of humans or animals.

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