PEARLS

A call to arms: Mustering secondary metabolites for success and survival of an opportunistic pathogen

Nicholas Raffa, Nancy P. Keller

1 Department of Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin, United States of America, 2 Department of Bacteriology, University of Wisconsin-Madison, Madison, Wisconsin, United States of America

* npkeller@wisc.edu

Introduction

Aspergillus fumigatus is a ubiquitous saprophytic mold able to grow on a diversity of material ranging from decayed organic matter in the environment to space station cupolas [1]. Yet this fungus is equally adept as a serious opportunistic pathogen, causing pulmonary aspergillosis and the more deadly invasive aspergillosis (IA). There are an estimated 3,000,000 cases of pulmonary aspergillosis annually and more than 200,000 cases of IA each year reaching a mortality rate of up to 90% in the most susceptible populations [2]. Difficulties in treating IA include delayed detection and increasing resistance to antifungal treatment. Like many opportunistic fungi, there is no one gene that makes A. fumigatus such a threatening pathogen. One unique feature of this pathogen is its arsenal of small molecules that impact disease development. Secondary metabolites are characterized as bioactive molecules of low molecular weight that are not required for growth of the organism but instead aid survival in harsh environments, resisting desiccation and UV stress and improving competition with other microbes. For A. fumigatus, these benefits extend to aiding growth not only in the environment but in the human body as well. Some secondary metabolites combat the host immune system by affecting immune cell function or by shielding the fungus against host attack, whereas others allow the fungus to acquire essential, scarce cofactors. The following synopsis of secondary metabolites produced by the opportunistic human pathogen A. fumigatus highlights how microbial metabolites, although undoubtedly evolved as environmental protectants, can impact infectious disease development (Fig 1). Although we delineate the roles of each metabolite by category for ease of discussion (e.g., “on the offensive,” “scavenging the battlefield,” “arms race”), the reader should note that each metabolite may have several biological roles for the fungus, in part illustrated in Fig 1.

On the offensive: How A. fumigatus combats the immune system

Once inside the host, A. fumigatus must survive interactions with components of the immune system by avoiding, suppressing, or weakening the immune response. The following secondary metabolites have been shown to impact disease or interactions with the immune system through such mechanisms.

Dihydroxynaphthalene melanin

Dihydroxynaphthalene (DHN) melanin is a polymer consisting of 1,8-dihydroxynaphthalene, found on the conidial surface. As an environmental benefit, DHN melanin helps to prevent...
Roles of *Aspergillus fumigatus* Secondary Metabolites

**Host**
- Iron Homeostasis
- Iron Acquisition
- Copper Homeostasis
- Inhibitor of Phagocytic Activity
- Cilostatin Inhibitor
- Acyltransferase Inhibitor

**Environment**
- Ferricron
- Hexadehydroactinone
- Triserythrobinone C
- Dihydropyrophosphate (Melanin)
- Helvolic Acid
- Pyrethroids A

---

**Host**
- Lipid Homeostasis
- Iron Homeostasis
- Copper Homeostasis

**Environment**
- Antiprotease
- Trypactin
- Antiprotectase
- Fumagillin
- Fumiquinazoline
- Nelauracillin

---

**Host**
- Antiphagocytic
- Reduces TNF-α Production
- Inhibits Neutrophil Chemotaxis

**Environment**
- Endocerin
- Protection Against UV Stress
- Antifungal/Antifeedant
- Antibacterial
- Inhibitor of Bacterial Replication
- Immune Response Inhibitor

---

**Host**
- Protection Against ROS and Phagosomal Acidification
- Glutathione

**Environment**
- Pseudotinin
- Fumiquinazoline
- Inhibitors of Bacterial Replication

---

**Host**
- Protease Inhibitory

**Environment**
- Unknown
- Unknown
desiccation of spores and confers resistance to UV radiation [3]. In the host, DHN melanin protects the conidia by scavenging reactive oxygen species [4], reducing phagosomal acidification in alveolar macrophages [5], and inhibiting apoptosis in epithelial cells [6]. When the polyketide synthase gene \( \text{pksP/} \text{alb1} \) responsible for the initial step of melanin production is deleted, there is a loss of spore pigment, a defect in virulence in intravenously injected immunocompetent murine models, and rapid killing spores in macrophage models [4]. Recently, DHN melanin has been described as a pathogen-associated molecular pattern, in that a C-type lectin receptor expressed in myeloid cells and CD31+ endothelial cells in humans recognizes DHN melanin and has been shown to have a protective role against disseminated infection in immunocompetent mice and recipients of stem cell transplants [7].

Gliotoxin

Gliotoxin is an epidithiodioxopiperazine that has been extensively studied in the context of infection. Gliotoxin inhibits activity of proteins that contain susceptible free thiols such as the host NADPH oxidase, a protein complex necessary for the generation of antimicrobial reactive oxygen species [8]. Gliotoxin has also been shown to inhibit nuclear factor-kappa B (NF-kB)-mediated transcription of cytokine genes and decrease cytotoxic activities of T lymphocytes [9]. \( A. \text{fumigatus} \) is resistant to its own toxin through a protective enzyme encoded in the gliotoxin cluster [10]. More recently, this metabolite has been shown to suppress the macrophage immune response by preventing integrin activation, interfering with actin dynamics, and impairing phagocytosis through affecting phosphoinositide metabolism [11]. When the gliotoxin nonribosomal peptide synthetase gene, \( \text{gliP} \), is deleted, there is an attenuation of virulence in non-neutropenic murine models of IA but not in neutropenic murine models [12].

Endocrocin

Endocrocin is a polyketide that is localized to the conidia during growth [13]. Using an in vivo zebrafish assay, endocrocin was found to directly affect immune cells by inhibiting neutrophil chemotaxis [14]. When the polyketide synthase gene \( \text{encA} \) is deleted, there is an attenuation of virulence using the \( Drosophila \text{ melanogaster} \) IA model [15]. Endocrocin belongs to a common class of anthraquinones and is closely related to emodin, a precursor in the trypacidin pathway that has been associated with mediating neutrophil apoptosis [15]. Although an exact role for endocrocin has not been established in nature, several related metabolites provide UV protection to fungi, similar to the role of DHN melanin [3].

Fumagillin

Fumagillin is a monoterpenoid, amoebicidal toxin with valuable pharmaceutical potential due to its inhibitory activity against methionine aminopeptidase-2, making it useful for the treatment of microsporidiosis [16]. The toxin has been found to suppress the immune response of \( Galleria \text{ mellonella} \) by inhibiting the activity of phagocytes [17] and reduces the ability of the insect immune cells to kill opsonized \( \text{Candida albicans} \) cells and phagocytose \( A. \text{fumigatus} \) conidia [17]. In addition, fumagillin also reduces the ability of hemocytes to take up oxygen and inhibits the translocation of p47 protein [17], an essential component of the NADPH
Fumagillin administered to insect larvae increases the susceptibility of the larvae to *A. fumigatus* [18]. Recently, virulence assays with an *A. fumigatus* fumagillin deletion mutant strongly support a role for this toxin in epithelial cell damage during IA [19].

**Fumigaclavines**

Fumigaclavines are ergot alkaloids, a class of compounds known to act as feeding deterrents and exhibit insecticidal and bactericidal activities [20]. Using the *G. mellonella* insect model for IA, it was found that a strain of *A. fumigatus* deficient in all ergot alkaloid production, ΔdmaW, resulted in a significantly reduced virulence. Strains that were still able to produce ergot alkaloids, but not fumigaclavine C, were significantly less virulent than wild type but still more virulent than the strain in which there was no production of ergot alkaloids, suggesting a role of the end product fumigaclavine C in virulence [20]. Fumigaclavine C has also been shown to inhibit the production of the pro-inflammatory cytokine tumor necrosis factor alpha (TNFα), suggesting a mechanism of action for the molecule [21].

**Scavenging the battlefield: How *A. fumigatus* acquires essential micronutrients**

Secondary metabolites regulate key aspects of micronutrient homeostasis and allow *A. fumigatus* to continue normal cellular function by meeting the needs for the trace elements such as copper and iron. Both are toxic in high doses but are necessary for essential cellular processes such as respiration and branched-chain amino acid biosynthesis. The ability to acquire these micronutrients is directly related to the ability of *A. fumigatus* to cause disease.

**Siderophores**

Siderophores produced by *A. fumigatus* are characterized by their hydroxamate moieties and function in high-affinity iron uptake and storage mechanisms. Extracellular siderophores fusaridine C and triacetlyfusaridine C are secreted into the environment, where they bind Fe³⁺ and transport it back into the cell. Intracellular siderophores ferricrocin and hydroxyferricrocin are responsible for iron storage and homeostasis. When the enzyme responsible for the first step in siderophore biosynthesis sidA is deleted, both extracellular and intracellular siderophore production is abolished. The sidA deletion grows poorly under iron-limiting conditions [22] and displays increased sensitivity to hydrogen peroxide. In addition, this mutant was found to be highly attenuated in virulence using a neutropenic murine model [23], suggesting that proper iron acquisition is essential for disease progression in the host.

**Hexadehydroastechrome**

Hexadehydroastechrome (HAS) is a tryptophan-derived secondary metabolite that binds to iron. Overexpressing the transcription factor present within the HAS biosynthetic gene cluster results in an increase in both siderophore and HAS production in addition to increased virulence in a neutropenic murine model [24]. HAS regulates fungal iron homeostasis circuitry, aligning iron acquisition and consumption pathways with secondary metabolite expression [25], including the newly discovered xanthocillin gene cluster [26].

**Xanthocillins**

Xanthocillins are tyrosine-derived metabolites that contain a characteristic isocyanide functional group and have been recently shown to be produced by the xan cluster in *A. fumigatus*. Overexpression of the transcription factor present within the cluster results in an increased
production of isocyanides and a defect in copper-dependent pigmentation indicating a possible link of this cluster to copper homeostasis [26]. The isocyanides produced by \textit{A. fumigatus} may represent a unique mechanism, on top of the canonical copper regulatory system [27], to maintaining copper homeostasis for this pathogen.

\textbf{Arms race: How \textit{A. fumigatus} uses secondary metabolites to compete in the environment and host}

Several secondary metabolites have no known effect or have not been tested for effects on virulence or interactions with the immune system but have only been shown to provide an advantage to \textit{A. fumigatus} when competing with other microbes in the environment.

\textbf{Trypacidin}

Trypacidin is an anthraquinone that has been found to have antiprotozoal, cytotoxic, and antiphagocytic properties. The compound displays activity against \textit{Toxoplasma gondii} and \textit{Trypanosoma cruzi} in vitro that causes toxoplasmosis and Chagas disease, respectively. Deleting the polyketide synthase essential for trypacidin production eliminates production of the metabolite and coincides with an increase in phagocytosis when challenged with \textit{Dictyostelium discoideum} and macrophages, indicating that trypacidin acts as an antiphagocytic metabolite [28]. The trypacidin pathway shows redundant synthesis to the endocrocin pathway, where both contribute to final endocrocin synthesis in some strains of \textit{A. fumigatus} [15].

\textbf{Helvolic acid}

Helvolic acid is a fusidane antibiotic that exhibits in vitro antiprotozoal activity against the trypanosome \textit{Trypanosoma brucei} GUTat3.1, the causative agent of African sleeping sickness [29], and helvolic acid derivatives exhibit antibacterial activity against \textit{Streptococcus agalactiae} and \textit{Staphylococcus aureus} [30]. In addition, helvolic acid also affects mammalian cell lines, decreasing the beat frequency of ciliated respiratory epithelium, a process important in preventing colonization by \textit{A. fumigatus} [31].

\textbf{Fumiquinazolines}

Fumiquinazolines are tryptophan-derived peptidyl alkaloids that have a broad range of activity and accumulate in \textit{A. fumigatus} conidia [32]. Fumiquinazoline F isolated from cultures of \textit{Penicillium coryphilum} exhibited activity against \textit{S. aureus} and \textit{Micrococcus luteus} [33]. Fumiquinazolines also exhibit antifungal activity with fumiquinazoline H and I isolated from \textit{Acremonium sp}. showing weak antifungal activity against \textit{C. albicans} [34].

\textbf{Fumitremorgins}

Fumitremorgins belong to the diketopiperazine alkaloids class of compounds and contain a unique, 8-membered endoperoxide ring. Fumitremorgin B has been found to have in vitro antifungal activity against a variety of phytopathogenic of fungi [35]. In addition, fumitremorgin B was found to be lethal to brine shrimp and displayed antifeedant activity towards armyworm larvae [35]. Fumitremorgins have also been shown to affect mammalian cells. Fumitremorgin C displays inhibitory activity towards the breast cancer resistance protein, an ATP-binding cassette transporter that is implicated in cellular resistance to anticancer drugs [36].
Pyripyropene A

Pyripyropene A was discovered during an investigation into inhibitors of acyl-coenzyme A (CoA):cholesterol acyltransferase, a mechanism by which to treat hypercholesterolemia and atherosclerosis [37]. Pyripyropenes were further shown to exhibit in vivo aphidicidal activity against the green peach aphid (Myzus persicae) during a screen of compounds that act as insecticides [38]. How these activities may relate to aspergillosis has not been assessed.

Pseurotin

Pseurotin has been shown to have several antimicrobial and cytotoxic properties. It has been demonstrated to have antibacterial properties when screened against both gram-positive and gram-negative organisms [39]. This metabolite is encoded by an intertwined biosynthetic gene cluster with fumagillin [40] but, unlike fumagillin, was not implicated in epithelial tissue damage [19].

Neosartoricin

Neosartoricin is a prenylated anthracenone and was discovered following activation of the gene cluster from A. fumigatus and Neosartorya fischeri [41]. The compound was found to have T-cell antiproliferative activity suggesting that the compound functions as an immunosuppressive [41]. Like several metabolites synthesized by A. fumigatus, the biosynthetic gene cluster is conserved in several pathogenic fungi [42].

Fumisoquin

Fumisoquin is an isoquinolone alkaloid with biosynthetic machinery that bears a striking similarity to plant berberine bridge enzyme and tetrahydrocannabinol biosynthesis [43]. Deletion of the fumisoquin transcription factor did not impact virulence in a murine infection model [44]. A related isoquinalone metabolite produced by Aspergillus flavus stimulates Aspergillus species spore germination while inhibiting bacterial growth [45], possibly hinting at a function for fumisoquin.

Nidulanin A

Nidulanin A is a tetracyclopeptide/isoprene isolated from Aspergillus nidulans [46]. The nidulanin A gene cluster is conserved in all Aspergillus spp., including A. fumigatus, although it has not been detected in this fungus [42]. At present, nidulanin A has yet to be tested for any antimicrobial or virulence-related properties.

Prospective

A. fumigatus produces a wide variety of small molecules, many of which are demonstrated to impact virulence, others of which have not been investigated, and likely still some of which have yet to be discovered. These molecules are the weapons that A. fumigatus uses to do battle with the immune system, facilitate the acquisition of essential micronutrients in their environment, and compete with other microbes. It is important to note, however, that A. fumigatus isn’t alone in producing secondary metabolites that affect virulence. Many of these secondary metabolites are conserved in other pathogenic fungi [38]. Studying secondary metabolites produced by A. fumigatus will provide insight into understanding not only the chemical arsenal of A. fumigatus but the chemical arsenal of other pathogenic fungi as well.
References

1. Knox BP, Blachowicz A, Palmer JM, Romsdahl J, Huttenlocher A, Wang CC, Keller NP, Venkateswaran K. Characterization of Aspergillus fumigatus isolates from air and surfaces of the international Space Station. mSphere. 2016 Oct 26; 1(5):e00227–16.

2. Taccone FS, Van den Abeele AM, Bulpa P, Messerseman W, Cardoso T, Paiva JA, Blasco-Navalpoto M, De Laere E, Dimopoulos G, Rello J. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation, underlying conditions, and outcomes. Crit Care. 2015 Dec; 19(1):7.

3. Nguyen KH, Chollet-Krugler M, Gouault N, Tomasi S. UV-protectant metabolites from lichens and their symbiotic partners. Nat Prod Rep. 2013; 30(12):1490–508. https://doi.org/10.1039/c3np70064j PMID: 24170172

4. Brakhage AA, Liebmann B. Aspergillus fumigatus conidial pigment and cAMP signal transduction: significance for virulence. Medical mycology. 2005 Jan 1; 43(1):75–82.

5. Jahn B, Langfelder K, Schneider U, Schindel C, Brakhage AA. PKSP-dependent reduction of phagolysosome fusion and intracellular kill of Aspergillus fumigatus conidia by human monocyte-derived macrophages. Cell microbiol. 2002 Dec; 4(12):793–803. PMID: 12484010

6. Berkova N, Lair-Fulleringer S, Féminéa F, Huet D, Wagner MC, Gorna K, Ibrahim-Granello N, Langfelder K, Schneider U, Schindel C, Brakhage AA. PKSP-dependent reduction of phagolysosome fusion and intracellular kill of Aspergillus fumigatus conidia by human monocyte-derived macrophages. Cell microbiol. 2002 Dec; 4(12):793–803. PMID: 12484010

7. Schlam D, Cantor J, Carreño M, Kopinski H, Freeman SA, Grinstein S, Faim GD. Gliotoxin suppresses macrophage immune function by subverting phosphatidylinositol 3, 4, 5-trisphosphate homeostasis. MBio. 2016 May 4; 7(2):e02242–15. https://doi.org/10.1128/mBio.02242-15 PMID: 27048806

8. Scharf DH, Brakhage AA, Mukherjee PK. Gliotoxin–bane or boon?. Environ Microbiol. 2016 Apr; 18(4):1096–109. https://doi.org/10.1111/1462-2920.13080 PMID: 26443473

9. Yamada A, Kataoka T, Nagai K. The fungal metabolite gliotoxin: immunosuppressive activity on CTL-mediated cytotoxicity. Immunol Lett. 2000 Jan 10; 71(1):27–32. PMID: 10709782

10. Dolan SK, O’Keeffe G, Jones GW, Doyle S. Resistance is not futile: gliotoxin biosynthesis, functionality and utility. Trends Microbiol. 2015 Jul 1; 23(7):419–28. https://doi.org/10.1016/j.tim.2015.02.005 PMID: 25766143

11. Martin J, Decker H, Schuchardt F, Beier K, Langfelder K, Schneider U, Schindel C, Brakhage AA. Mechanism of gliotoxin-induced stimulation of human primary macrophages. J Biol Chem. 2006 Feb 24; 281(9):5303–9. https://doi.org/10.1074/jbc.M511056200 PMID: 16457811

12. Dagenais TR, Keller NP. Pathogenesis of Aspergillus fumigatus in invasive aspergillosis. Clin Microbiol Rev. 2009 Jul 1; 22(3):447–65. https://doi.org/10.1128/CMR.00055-08 PMID: 19597008

13. Lim FY, Hou Y, Chen Y, Oh JH, Lee I, Bugni TS, Keller NP. Genome-based cluster deletion reveals endocrinin biosynthetic pathway in Aspergillus fumigatus. Appl Environ Microbiol. 2012 Apr 6;AEM-07710.

14. Berthier E, Lim FY, Deng Q, Guo CJ, Kontoyiannis DP, Wang CC, Rindy J, Beebe DJ, Huttenlocher A, Keller NP. Low-volume toolbox for the discovery of immunosuppressive fungal secondary metabolites. PLoS Pathog. 2013 Apr 11; 9(4):e1003288.

15. Throckmorton K, Lim FY, Kontoyiannis DP, Zheng W, Keller NP. Redundant synthesis of a conidial polyketide by two distinct secondary metabolite clusters in Aspergillus fumigatus. Environ microbiol. 2016 Jan; 18(1):246–59. https://doi.org/10.1111/1462-2920.13007 PMID: 26242966

16. Mendoza Y, Diaz-Cetti S, Ramallo G, Santos E, Pornini M, Invernizzi C. Nosema ceranae Winter Con-symbiotic partners. Nat Prod Rep. 2013; 30(12):1490–508. https://doi.org/10.1039/c3np70064j PMID: 24170172

17. Fallon JP, Reeves EP, Kavanagh K. Inhibition of neutrophil function following exposure to the Aspergil-lius fumigatus toxin fumagillin. J Med Microbiol. 2010 Jun 1; 59(6):625–33.

18. Fallon JP, Reeves EP, Kavanagh K. The Aspergillus fumigatus toxin fumagillin suppresses the immune response of Galliera mellonella larvae by inhibiting the action of haemocytes. Microbiology. 2011 May 1; 157(5):1481–8.

19. Guruceaga X, Ezpeleta G, Mayayo E, Sueiro-Olivares M, Abad-Díaz-de-Cerio A, Aguirre JM, Liu HG, Wiemann P, Bok JW, Filler SG, Keller NP, Hernando FL, Ramirez-Garcia A, Rementeria A. A possible role for fumagillin in cellular damage during host infection by Aspergillus fumigatus. Virulence. 2018 Sep 24; 9(1):1548–1561. https://doi.org/10.1080/21505594.2018.1526528 PMID: 30251593

20. Panaccione DG, Arnold SL. Ergot alkaloids contribute to virulence in an insect model of invasive aspergillosis. Sci Rep. 2017 Aug 21; 7(1):8930. https://doi.org/10.1038/s41598-017-09107-2 PMID: 28827626
21. Du RH, Li EG, Cao Y, Song YC, Tan RX. Fumigacaine C inhibits tumor necrosis factor α production via suppression of toll-like receptor 4 and nuclear factor-kB activation in macrophages. Life Sci. 2011 Aug 15; 89(7–8):235–40. https://doi.org/10.1016/j.lfs.2011.06.015 PMID: 21762706

22. Hissen AH, Wan AN, Warwas ML, Pinto LJ, Moore MM. The Aspergillus fumigatus siderophore biosynthetic gene sidA, encoding L-ornithine N5-oxygenase, is required for virulence. Infect Immun. 2005 Sep 1; 73(9):5493–503. https://doi.org/10.1128/IAI.73.9.5493-5503.2005 PMID: 16113265

23. Schreitl M, Bignell E, Kraig C, Joechi C, Rogers T, Arst HN, Haynes K, Haas H. siderophore biosynthesis but not reductive iron assimilation is essential for Aspergillus fumigatus virulence. J Exp Med. 2004 Nov; 200(9):1213–9. https://doi.org/10.1084/jem.20041242 PMID: 15504822

24. Yin WB, Baccile JA, Bok JW, Chan Y, Keller NP, Schroeder FC. A nonribosomal peptide synthetase-derived iron (III) complex from the pathogenic fungus Aspergillus fumigatus. J Am Chem Soc 2013 Feb 1; 135(6):2064–7. https://doi.org/10.1021/ja311145n PMID: 23360537

25. Wiemann P, Lechner BE, Baccile JA, Velk TA, Yin WB, Bok JW, Chen Y, Keller NP, Schroeder FC, Haas H. Perturbations in small molecule synthesis uncovers an iron-responsive secondary metabolite network in Aspergillus fumigatus. Front Microbiol. 2014 Oct 24; 5:530. https://doi.org/10.3389/fmicb.2014.00530 PMID: 25386169

26. Lim FY, Won TH, Raffa N, Baccile JA, Wisecaver J, Rokas A, Schroeder FC, Keller NP. Fungal Isocynoamide Synthases and Xanthochillin Biosynthesis in Aspergillus fumigatus. mBio. 2018 Jul 5; 9(3):e00785–18. https://doi.org/10.1128/mBio.00785-18 PMID: 29844112

27. Wiemann P, Perevitsky A, Lim FY, Shadkchan Y, Knox BP, Figueora JA, Choera T, Niu M, Steinerberger AJ, Wüthrich M, Idol RA. Aspergillus fumigatus copper export machinery and reactive oxygen intermediate defense counter host copper-mediated oxidative antimicrobial offense. Cell Rep. 2017 May 2; 19(5):1008–21. https://doi.org/10.1016/j.celrep.2017.04.019 PMID: 28407895

28. Mattern DJ, Schoeler H, Weber J, Novohradská S, Kralbooc K, Dahse HM, Hillmann F, Valiante V, Figge MT, Brakhage AA. Identification of the antiphagocytic tryptacin gene cluster in the human-pathogenic fungus Aspergillus fumigatus. Appl Microbiol Biotechnol. 2015 Dec 1; 99(23):10151–61. https://doi.org/10.1007/s00253-015-6898-1 PMID: 26278536

29. Goto K, Horikoshi R, Mitomi M, Oyama K, Hirose T, Sunazuka T, Omura S, Tomoda H, Kim YK, Nishida H. Pyripyropenes, highly potent inhibitors of acyl-CoA: cholesterol acyltransferase produced by Aspergillus fumigatus. J Antibiot (Tokyo). 1993 Jul; 46(7):1168–89.

30. Goto K, Horikoshi R, Milomi M, Oyama K, Hirose T, Sunazuka T, Omura S. Synthesis and insecticidal efficacy of pyripyropene derivatives focusing on the C-1, C-7, and C-11 positions’ substituent groups. J Antibiot (Tokyo). 2018 May 23: 1.
39. Mehedi MA, Molla AH, Khondkar PR, Sultana S, Islam MA, Chowdhury R. Pseurotin A: an antibacterial secondary metabolite from Aspergillus fumigatus. Chem Asian J. 2010 Apr 1; 22:2611–4.

40. Wiemann P, Guo CJ, Palmer JM, Sekonyela R, Wang CC, Keller NP. Prototype of an intertwined secondary-metabolite supercluster. Proceedings of the National Academy of Sciences. 2013 Oct 15; 110 (42):17065–70.

41. Yin WB, Chooi YH, Smith AR, Cacho RA, Hu Y, White TC, Tang Y. Discovery of cryptic polyketide metabolites from dermatophytes using heterologous expression in Aspergillus nidulans. ACS Synth Biol. 2013 Jun 11; 2(11):629–34. https://doi.org/10.1021/sb400048b PMID: 23758576

42. Bignell E, Cairns TC, Throckmorton K, Nierman WC, Keller NP. Secondary metabolite arsenal of an opportunistic pathogenic fungus. Phil Trans R Soc B. 2016 Dec 5; 371(1709):20160023. https://doi.org/10.1098/rstb.2016.0023 PMID: 28080993

43. Baccile JA, Spraker JE, Le HH, Brandenburger E, Gomez C, Bok JW, Macheleidt J, Brakhage AA, Hoffmeister D, Keller NP, Schroeder FC. Plant-like biosynthesis of isoquinoline alkaloids in Aspergillus fumigatus. Nat Chem Biol. 2016 Jun 1; 12(6):419–24. https://doi.org/10.1038/nchembio.2061 PMID: 27065235

44. Macheleidt J, Scherlach K, Neuwirth T, Schmidt-Heck W, Straßburger M, Spraker J, Baccile JA, Schroeder FC, Keller NP, Heinekamp T. Transcriptome analysis of cyclic AMP-dependent protein kinase A–regulated genes reveals the production of the novel natural compound fumipyrrole by Aspergillus fumigatus. Mol Microbiol. 2015 Apr; 96(1):148–62. https://doi.org/10.1111/mmi.12926 PMID: 25582336

45. Khalid S, Baccile JA, Spraker JE, Tannous J, Imran M, Schroeder FC, Keller NP. NRPS-derived isoquinolines and lipopeptides mediate antagonism between plant pathogenic fungi and bacteria. ACS Chem Biol. 2017 Dec 18; 13(1):171–8. https://doi.org/10.1021/acschembio.7b00731 PMID: 29182847

46. Andersen MR, Nielsen JB, Kirtggaard A, Petersen LM, Zachariassen M, Hansen TJ, Blicher LH, Gottfredsen CH, Larsen TO, NielsenKF, Mortensen UH. Accurate prediction of secondary metabolite gene clusters in filamentous fungi. Proceedings of the National Academy of Sciences. 2013 Jan 2; 110(1):E99–107.