REVIEW ARTICLE

A Systematic review of *Aspergillus fumigatus* metabolites of antimicrobial importance

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ABSTRACT

Background: *Aspergillus fumigatus* is a common fungus in many environments; from the air we breathe and the ground beneath us to even the International Space Station. It has proven capable of yielding no fewer than 226 bioactive compounds, 36 of which are chemical structures that may be connected to the genome of *Aspergillus fumigatus* through biosynthetic gene clusters (BGCs). This systematic review aims to investigate the status of *Aspergillus fumigatus* metabolites that are of antimicrobial importance. **Methods**: PubMed was systematically searched on November 5, 2023, using the algorithm "Aspergillus fumigatus" AND "Metabolites" AND "Antimicrobial." In line with PRISMA guidelines, three qualifying studies were selected for inclusion. **Results**: A total of sixty (60) articles were retrieved without duplicates, of which forty-eight (48) articles were screened out upon title and abstract screening. Five articles (5) were also excluded for lack of accessible full text, and finally four (4) articles were removed as review articles, and three (3) studies were used in this research as they meet the criteria since the review is focusing on only experimental work that studied metabolites of *Aspergillus fumigatus*with antimicrobial properties with no restriction to year or continent. The three articles included in the study revealed 77 metabolites, among which 29 are polyketides, 26 are terpenoids, 14 are peptides, and 9 are alkaloids, as they were classified based on structural and biochemical characteristics. These metabolites exhibit broad-spectrum antimicrobial effects and offer promising templates for the development of novel drugs. **Conclusion**: In our review, we highlighted secondary metabolites of *Aspergillus fumigatus*from different environments around the world that demonstrated antimicrobial effects. This has not been reported in any previous work as far as we know, as previous publications reported the genus generally, but this work is specific to the fumigatus species, and it needs to be explored in different environments, especially extreme environments like hot springs, where there is less information available. Previous research has demonstrated that microorganisms from extreme environments are excellent candidates to produce antimicrobials.

Keywords: *Aspergillus fumigatus*, Metabolites, Antimicrobial

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INTRODUCTION

The continuous emergence of drug-resistant microorganisms poses a major global health concern that requires the discovery of novel antimicrobial agents(1). Natural products have long been a cornerstone in this search, particularly those derived from fungi, which naturally generate many different secondary metabolites with biological effects(2). Filamentous fungi of the genus Aspergillus have attracted significant attention because of their biosynthetic potential, and among them, *Aspergillus fumigatus*holds a dual identity: both as a major opportunistic human pathogen and a prolific producer of chemically diverse secondary metabolites(3).

A. fumigatus is one of the most prevalent airborne fungal species, capable of surviving in a variety of environmental conditions(4). It is commonly associated with serious infections in immunocompromised individuals, such as invasive aspergillosis(5). Despite its clinical notoriety, *A. fumigatus* is also an important model organism for studying fungal metabolism due to its well-characterized genome and amenability to genetic manipulation(3).

Secondary metabolites from *A. fumigatus* are synthesized via complex biosynthetic pathways involving non-ribosomal peptide synthetases (NRPS), polyketide synthases (PKS), and hybrid enzymes(6). These metabolites are not essential for the fungus's growth but serve ecological functions like competition, communication, and survival under stress(7). Some of these compounds, including gliotoxin, fumagillin, pseurotin A, and Helvolic acid, have demonstrated potent antimicrobial effects against a variety of harmful bacteria and fungi(8).

The antimicrobial activity of *A. fumigatus* metabolites is of particular interest in the context of drug resistance, as these natural compounds may serve as scaffolds for the development of new antibiotics or antifungals(9). Furthermore, advances in omics technologies and bioinformatics have facilitated the finding of previously unknown biosynthetic gene clusters (BGCs) in A. fumigatus, pointing to the existence of many yet-undiscovered metabolites with possible pharmacological relevance(9).

Given the urgency of addressing antimicrobial resistance and the underexplored chemical diversity of A. fumigatus, a systematic review of its antimicrobial metabolites is both timely and essential. This review aims to gather, evaluate, and synthesize current research findings on the antimicrobial secondary metabolites produced by *A. fumigatus* to support future efforts in natural product drug discovery.

Materials and Methods

Search Strategy

We searched the PubMed database on the 5th of November, 2023, to identify studies on the antimicrobial activity of metabolites of Aspergillus fumigatus. The search was run using the algorithm "Aspergillus fumigatus" AND "metabolites" AND "antimicrobial" with no restriction to study side or year of publication. No protocol was registered before the commencement of this study.

Study Selection Criteria

Inclusive criteria

Study area: No restriction on to study area in this review.

Study design: Only experimental studies that isolated, identified, and tested the antimicrobial activities of *A. fumigatus* were considered in this review.

Language: All the studies used in this review were written in the English language.

Publication issue: Only papers published in PubMed were considered in this review. It was chosen as the primary database for this systematic review due to its comprehensive coverage, credibility, and relevance to the biomedical and life sciences fields(10). Furthermore, it provides access to over 35 million citations from a wide range of peer-reviewed journals, including those focused on microbiology, pharmacology, biotechnology, and natural products.

Exclusive criteria

Non-experimental studies, experimental studies focusing on non-antimicrobial activities (e.g., anti-inflammatory, anticancer, or antidiabetic effects), as well as experimental studies isolating Aspergillus species other than A. fumigatus, even if their metabolites were assessed for antimicrobial activity, were excluded.

Data Extraction

Search results from the database were screened by title and abstract against the eligibility criteria. Full-text articles were then assessed for eligibility, and each exclusion was noted along with its justification. Both the title/abstract and full-text screenings were conducted independently by two reviewers (K.A.L. and U.Y.U.). Any disagreements were settled by a third reviewer (S.A.A.).

(Supplementary material 1).

Results

Study Selection and Characteristics

The included studies were limited to experimental research

investigating the antimicrobial activity of metabolites produced by *Aspergillus fumigatus*isolated from plant, marine, and terrestrial sources. A total of 60 records were retrieved from PubMed, with no duplicates identified. During title and abstract screening, 48 articles were excluded. An additional five articles were excluded due to a lack of full-text availability, and four were removed as they were review articles. Ultimately, three studies met the criteria and were included in this review, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Figure 1).

*Aspergillus fumigatus*metabolites

*Aspergillus fumigatus*is known to produce a diverse array of secondary metabolites spanning multiple classes, including polyketides, phenolics, triterpenes, sterols, sesquiterpenes, alkaloids, and fatty acids(11). Several studies have documented these metabolites, along with the extraction techniques and solvents used, which are summarized in this review (Table 1).

Antimicrobial activity of *Aspergillus fumigatus*metabolites

*Aspergillus fumigatus*is renowned for its prolific production of bioactive compounds, several of which exhibit significant antimicrobial effects (12). These bioactive compounds are synthesized through complex biosynthetic pathways and have been isolated from various sources, including soil, plant tissues, and marine environments (13). The antimicrobial potential of these metabolites differs according to their chemical structure, the source of the fungal isolate, and the cultivation and extraction methods employed (12).

Antimicrobial activity of the most Notable Antimicrobial Metabolites of *Aspergillus fumigatus* identified in this review are described below, and (Table 2.

Helvolic Acid

Helvolic acid, a polyketide compound, showed strong antibacterial effects against Gram-positive bacteria, including Staphylococcus aureus and even the drug-resistant strain MRSA. In a study by Zhang et al. (14). Helvolic acid exhibited minimum inhibitory concentrations (MICs) of 3.12 µg/mL against S. aureus and MRSA, and 12.5 µg/mL against vancomycin-resistant Enterococcus species. The compound also showed activity against Shigella dysenteriae at a MIC of 100 µg/mL.

Ergosterol

Ergosterol, a sterol compound, was identified in a study by Hussein et al (15) as having antibacterial activity against S. aureus, the compound was isolated from an endophytic *A. fumigatus* strain derived from Albizia lucidior leaves. The study utilized ultra-performance liquid chromatography coupled with mass spectrometry (UPLC-MS/MS) to identify over 40 metabolites, with ergosterol being one of the active compounds.

Monomethyl Sulochrin-4-Sulphate

This compound is also isolated from the same *A. fumigatus* strain exhibited a MIC of 3.90 µg/mL against S. aureus. Molecular docking studies indicated that Monomethyl Sulochrin-4-sulfate may block the action of DNA gyrase and topoisomerase IV, two key enzymes that bacteria need to copy their DNA and reproduce (15).

N-formyl-4-hydroxyphenyl-acetamide and Atraric Acid

From a soil-derived *A. fumigatus* strain, two phenolic compounds were isolated: N-formyl-4-hydroxyphenyl-acetamide and Atraric acid, which showed powerful antifungal effects against several fungal strains, along with some power to combat multidrug-resistant bacterial infections. Atraric acid, in particular, was noted for its first-time isolation from a non-lichen fungal strain, suggesting potential for industrial-scale production(16).

Fumagillin

Fumagillin is a meroterpenoid compound first isolated from *A. fumigatus* in 1949. It has demonstrated antimicrobial activity, particularly against protozoan parasites. The compound's complex structure and mode of action continue to be subjects of research (8).

Discussions

This comprehensive review examines the diverse metabolites produced by Aspergillus fumigatus, isolated from terrestrial and marine environments worldwide, with special attention to their antibiotic potential. Regardless of geographic origin, the production of secondary metabolites by *A. fumigatus* generally follows a standardized workflow: isolation and identification of fungal strains, cultivation for metabolite biosynthesis, extraction of the produced compounds, purification, and subsequent physicochemical characterization. These key steps are outlined below

Culture of *Aspergillus fumigatus* for metabolites Extraction

Following the isolation and identification of Aspergillus fumigatus, the next step in metabolite production is cultivating the fungus. Typically, two primary fermentation methods are used for secondary metabolite (SM) biosynthesis: submerged fermentation (SmF) and solid-state fermentation (SSF). (17). SSF, carried out on solid substrates with low moisture content, enhances oxygen transfer and supports microbial growth. Solid substrates commonly include sugarcane bagasse, rice hulls, and rice straw. While previous studies have reported that SSF can

lead to high metabolite yields, the present review found that only one of the three included studies (33.3%) utilized this method, specifically using rice straw as a substrate. (12). In contrast, submerged fermentation (SmF) is more widely employed because it's easy to control key fermentation factors like pH, temperature, oxygen levels, and nutrient content. SmF was conducted in a liquid nutrient medium and was adopted in two out of the three reviewed studies (66.6%). Based on these findings, we are suggesting the use of submerged fermentation (SmF) in future research focused on the production of *Aspergillus fumigatus* metabolites, given its operational advantages and broader adoption. After fermentation, solvents are used for the extraction and purification of the metabolites. Choosing the right solvent is crucial for yield, purity, and safety.

Solvent use form of *Aspergillus fumigatus* metabolites production

The spectrum of metabolites obtained from *Aspergillus fumigatus*is is shaped not only by the cultivation method but also by the extraction strategy and choice of solvent. Common solvents employed for extracting metabolites from both submerged and solid-state cultures include ethyl acetate, dichloromethane, hexane, and chloroform. Among these, ethyl acetate has emerged as the most effective solvent, a conclusion supported by the findings of this review. Its consistent efficacy potentially resolves longstanding debates regarding optimal solvent selection for drug discovery from fungal isolates.

Ethyl acetate possesses several favorable chemical and biological properties, such as medium polarity, low toxicity to test organisms, and high volatility (18). Its moderate polarity allows for more efficient extraction of moderate non-polar compounds. (19) Furthermore, in the context of marine-derived fungi, ethyl acetate effectively separates organic compounds from water and salt, making it especially suitable for isolating pure organic metabolites (20). Due to these advantages, all the research examined in this review utilized ethyl acetate for the extraction of secondary metabolites Table 1.

Metabolites of *Aspergillus fumigatus*

Fungal metabolites are a diverse group of compounds that serve various essential functions, including roles in defense, intercellular communication, and nutrient acquisition, all of which are crucial for fungal survival (21). In this review, a total of 89 metabolites of *Aspergillus fumigatus* were identified. Of these, 45 were reported in research by Zhang et al., 42 in studies by Hussein et al., and 2 in work by Harman et al. Interestingly, further analysis revealed 77 distinct metabolites, as 12 metabolites were identified in multiple studies (Fumitremorgin C, Fumitremorgin B, Monomethyl Sulochrin, Chaetominine, Pseurotin A, Helvolic acid, Azaspirofuran B, Azaspirofuran A, Questin,, Pyripyropene A, Fumagiringillin and Fumigaclavine C) were found to overlap between the studies of Zhang et al. and Hussein et al., despite their distinct origins (marine and endophytic fungi, respectively). These metabolites were classified into four main categories based on their structural characteristics and biochemical properties: 29 polyketides, 14 peptides, 25 terpenoids, and 9 alkaloids, as outlined below.

The classification of fungal metabolites based on structure is highly relevant to understanding and predicting their antimicrobial activity (22). The structure informs us about the target site, potency, and spectrum of activity (e.g., bacteria vs fungi) (23). Different classes of fungal metabolites often interact with microbial cells in specific ways, as Polyketides (e.g. citrinin, lovastatin): Often inhibit enzymes or interfere with cell membranes Fig. 2. Peptides (e.g. cyclosporin, echinocandins) often inhibit cell wall synthesis or disrupt membranes Fig. 3. Terpenoids (e.g. sterols) disrupt microbial membranes due to their lipophilic nature Fig 4., Alkaloids (e.g. ergometrine): Can intercalate DNA or inhibit protein synthesis Fig 5.

Polyketides are large and structurally diverse compounds synthesized by polyketide synthases (PKSs) (19). This unique antimicrobial polyketide is made up of two different structural domains: a tetramic acid and a bicyclic hydrocarbon, joined by a carbonyl functional group (20). Tetramic acid derivatives found in both marine and terrestrial fungi, including the Arctic fungus from the Lindgomycetaceae family, have garnered significant attention because they have many different effects on living organisms and intricate structural complexity. Most of the compounds isolated so far have demonstrated primarily antibiotic, antiviral, and antifungal properties. Recognizing polyketides as potent antimicrobial agents, this review identifies twenty-nine (29) metabolites isolated from *A. fumigatus* across three reviewed articles, sourced from marine and terrestrial (endophytic and soil) fungi (Fig. 2) and their antimicrobial activity (Table 2). These metabolites include fumindoline A, fumindoline B, fumindoline C, 12β, 13β-Hydroxyasperfumigatin, Asperfumigatin, Demethoxyfumitremorgin C, fumitremorgin C, 12,13-Dihydroxyfumitremorgin C, 12a-Hydroxyoxofumitremorgin C, fumitremorgin B, 13-Oxofumitremorgin B, Fumiquinazoline C, Fumagiringillin, fumagillin, Sulochrin, dimethyl 2,30-

Dimethylosoate, questin, trypacidin, pyripyropene A, pyripyropene F, pyripyropene O, and 6,16-O-Dideacetyl helvolic acid. 21,16-lactone, 16-O-Deacetylhelvolic acid, 21,16-lactone, 16-O-propionyl-16-O-deacetylhelvolic acid/6-O-propionyl-6-O-deacetylhelvolic acid, 6-O-propionyl-6, 16-O-Dideacetylhelvolic acid 21,16-lactone, linoleic acid, oleic acid, 6-Methoxyspirotryprostatin B, and atraric acid.

Peptides: Fungi employ two distinct pathways for generating peptidic natural products. The nonribosomal peptide (NRP) pathway, orchestrated by enzymes, is responsible for the synthesis of most peptide metabolites (24). These complex, multi-part enzymes known as NRPSs use both standard and unusual amino acids to build molecules. The genes that produce these enzymes are usually found together in what's called a biosynthetic gene cluster, a group of closely linked and co-regulated genes (25). The alternative pathway involves ribosomally synthesized and post-translationally modified peptides (RiPPs), which yield notably large peptidic natural products with molecular weights typically around 1000 Da (26). This review identifies fourteen (14) fungal peptides from three articles included in the study, which isolated *A. fumigatus* from soil, marine, and endophytic fungi (Fig. 3) and their antimicrobial each (Table 2). These peptides have demonstrated significant value in cytotoxic properties and antibiotic drug discovery. They include Cyclotryprostatin B, Spirotryprostatin A, 11-epi-Chaetominine, Fumigaclavine B, Fumigaclavine C, Bisdethiobis(Methylthio)gliotoxin, Pseurotin F1, Pseurotin F2, Pseurotin A, 11-O-Methylpseurotin A, Penibenzophenone E, Cyclo-(Leu-Pro), Cyclo-(Phe-Pro), and Fumigatoside F.

Terpenoids: Terpenoids: Terpenoids represent a diverse group of natural compounds showing a variety of effects on living systems, including antifungal, antibacterial, and anticancer effects. They are synthesized from isoprene units (27). and are commonly associated with plant-derived natural products. However, several fungal species are also recognized for producing metabolites that come from terpenes. (28). Some compounds within the diterpene family, which consists of four isoprene units, are considered secondary metabolites. These diterpenoids show antibacterial effects against human pathogens, such as Staphylococcus aureus and Salmonella typhimurium (27). Likewise, some are acidic terpenoids that exhibit antibiotic activity against a range of bacterial, yeast, and fungal species, such as B. subtilis, Cryptococcus neoformans, C. albicans, and *A. fumigatus* (29). The broad-spectrum antimicrobial properties of these compounds are primarily attributed to their ability to inhibit glucan biosynthesis. Over the past decade, most bioactive fungal terpenes and terpenoids have been sourced from marine fungi and fungi associated with algae (12). This review identifies 25 terpenoids from A. fumigatus, isolated from marine and endophytic fungi (Fig. 4) and their antimicrobial activity shown in Table 2, including Verruculogen, 6-Methoxyspirotryprostatin B, Spirotryprostatin C, 2-Epi-Tryptoquivaline F, Helvolic acid, 6-O-propionyl-16-O-deacetylhelvolic acid, 16-O-propionyl-6-O-deacetylhelvolic acid, Monomethylsulochrin, 80-O-Methylasterric acid, (+)-20S-Isorhodoptilometrin, 6-hydroxy-8-methoxy -3-Methylisocoumarin, Isosclerone, 9-Deacetylfumigaclavine C, Cyclotryprostatin A, hexylitaconic acid, ethyl α-D-glucopyranoside, 5,8-Epidioxyergosta-6,9(11),22-trien-3-ol, ergosterol peroxide, (22E)-Ergosta-4,6,8(14),22,24(28)-Pentaen-3-one, Ergosta-4,6,8(14),22-tetraen-3-one, ergosterol, Methylorsilinate, Tryptoquivaline F, 9-Deacetoxyfumigaclavine C, Synerazol, and N-formyl-4-hydroxyphenyl-acetamide."

Alkaloids: Alkaloids constitute a vast category of naturally derived organic compounds, frequently possessing pharmacological impacts on humans and various animals (30). They commonly bear the suffix "-ine," a hallmark characteristic of alkaloids (31). While certain alkaloids, like the hallucinogenic LSD, have been employed as recreational substances, most alkaloids are linked to medicinal uses (32). These include treatments for migraine and tumors (such as ergotamine), as well as for conditions like Parkinson's disease or restless leg syndrome (33). However, what's less commonly recognized is their combined antimicrobial effectiveness. Lysergol 3, a synthetic alkaloid, demonstrates synergistic antibiotic properties, acting as a bioactive enhancer and facilitating the bioavailability of broad-spectrum antibiotics. This characteristic aids in the absorption of antibiotics through the cell membrane of animal cells, leading to heightened efficacy against both Gram-positive and Gram-negative bacteria (34). At the recommended lysergol dosage of 10 µg/mL, the antimicrobial effectiveness sees an enhancement ranging from 2 to 12 times against a broad spectrum of both Gram-positive and Gram-negative bacteria, including Escherichia coli, Bacillus subtilis, Mycobacterium smegmatis, and similar microorganisms (34). Recognizing the pharmaceutical significance of alkaloids, this review identifies nine (9) alkaloid metabolites from two studies isolating *A. fumigatus* from marine and endophytic fungi (Fig. 1), and their

antimicrobial activity is indicated in Table 2. These include (+)-Alantrypinone, Oxoglyantrypine, Chaetominine, Azaspirofuran B, Azaspirofuran A, Spirotryprostatin A, Spirotryprostatin C, Monomethylsulochrin-4-sulphate, and emodin.

Techniques for assessing antimicrobial activity of *A. fumigatus* metabolites

Several widely recognized and frequently used bioassays, such as the agar disc diffusion method, agar well diffusion method, microdilution method, and a method that incorporates the extract into the culture medium and measures the number of bacterial colonies, are used to evaluate the antibacterial activity of fungal extracts or pure compounds (35). As a result, the antibacterial activity data are represented in various units (36). Using the agar disc diffusion technique, varying quantities of the extract are added to discs (37). The diameter of the inhibition zone (IZD), which indicates antibacterial activity, was measured around each disc to determine the clear or inhibition zone that grew around it (38). The agar well diffusion method and the agar disc diffusion method work on the same concept; however, wells are used for a fixed amount of extract instead of discs (39). The microdilution method involves dilutions of the extract in a liquid medium using microplates to calculate the values of the minimum lethal concentration (MLC), minimum bactericidal concentration (MBC), or a concentration that inhibits 50% of the growth of bacteria (IC50) (39). In the final technique, the extract is mixed with the culture medium and the number of colony-forming units (CFU) that result is calculated (40). Some bioassays, such as flow cytofluorometric and bioluminescent techniques, are less common since they need specialized equipment and further testing to ensure consistency and repeatability. Balouiri et al. reviewed the aforementioned assessment techniques in detail. (41). The findings of this review have shown the utilization of only two of the above-mentioned, as Zhang et al. used the microdilution method, Harman et al. used the agar well diffusion method, and Hussein et al. combined the two (microdilution method and agar well diffusion), which is probably because of their advantages over the other assay methods mentioned above, as the disk-diffusion assay offers numerous advantages over other methods, including simplicity, low cost, the ability to test a large number of microorganisms and antimicrobial agents, and ease of result interpretation (42). Moreover, the agar disk-diffusion method is not suitable for determining the minimum inhibitory concentration (MIC) because it is impossible to quantify the amount of antimicrobial agent that has diffused into the agar medium (43). However, for certain microorganisms and antibiotics, an approximate MIC can be calculated by comparing the inhibition zones with stored algorithms (44). Dilution methods are considered the most suitable for determining MIC values, as they enable estimation of the concentration of the tested antimicrobial agent in either agar (agar dilution) or broth medium (macro dilution or microdilution). Although factors like inoculum size, growth medium, incubation time, and preparation method can influence the results, these methods have been standardized by CLSI for testing aerobically growing bacteria, yeast, and filamentous fungi (45). Therefore, the findings of this review suggest the use of agar well diffusion, microdilution, or both, depending on the target and available resources, because of their advantages over other methods, as mentioned above.

Comparison of *A. fumigatus* with Existing Antibiotics

A. fumigatus metabolites show promising and sometimes unique antimicrobial activity, but toxicity and limited development keep them behind existing antibiotics. However, their novel structures make them excellent candidates for new drug discovery, especially in the face of antibiotic resistance, as detailed in Table 3.

Conclusion

Over the years, various Aspergillus species have been isolated from different environments, with their metabolites being utilized for medicinal purposes. Review articles have been published on the medical significance of Aspergillus species from both marine and terrestrial environments. Among these species, *Aspergillus fumigatus*stands out for its notable antimicrobial potential. However, there has yet to be a dedicated review on the antimicrobial significance of A. fumigatus. This review aims to address this gap by highlighting studies that have isolated A. fumigatus, extracted its metabolites, and explored their antimicrobial properties. A total of three articles were retrieved from PubMed on 5/11/2023, all of which isolated *A. fumigatus* which are either from plant, marine, or soil environments. Notably, only one of these studies was conducted in Africa (Egypt), with no studies from East Africa. This underscores the need for further investigation of A. fumigatus, particularly from extreme environments such as hot springs, as the literature suggests that microorganisms from such habitats may uncover novel isolates that produce compounds with significant pharmaceutical and biotechnological potential.

Authors' contributions

In this study, KAL, SAA, and IAA. conceptualized and designed the research; K.A.L. retrieved data. KAL and UYU

screened the data, and K.A.L. drafted the initial manuscript. K.A.L. performed data interpretation. S.A.A., I.A.A., and U.Y.U. reviewed and edited the manuscript. All authors read and approved the final version for submission.

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Table 1: Various studies of the Metabolites of *A fumigatus*

Study	Metabolite	Method of Extraction	Solvent used
Zhang et al. (2022)	Fumindoline A, Fumindoline B, Fumindoline C, 12β, 13β-hydroxyasperfumigatin, Asperfumigatin, Demethoxyfumitremorgin C, Fumitremorgin C, 1,13-Dihydroxyfumitremorgin C,12*a*-hydroxy-13-oxofumitremorgin C (**9**), Fumitremorgin B, 13-oxofumitremorgin B, Cyclotryprostatin B, Verruculogen, 6-methoxyspirotryprostatin B, -Spirotryprostatin A, Spirotryprostatin C, 2-epi-tryptoquivaline F, Fumiquinazoline C, (+)-Alantrypinone, Oxoglyantrypine, Chaetominine, 11-epi-cChaetominine, Fumigaclavine C, Bisdethiobis(Methylthio)gliotoxin, Pyripyropene A, Pseurotin F1, Pseurotin F2, Pseurotin A, 11-O-Methylpseurotin A, Azaspirofuran B, Azaspirofuran A, Fumagiringillin, Fumagillin, Helvolic acid, 6-O-propionyl-16-O-Deacetylhelvolic acid, 16-O-propionyl-6-O-Deacetylhelvolic acid, Penibenzophenone E (37), Sulochrin, Monomethylsulochrin, 80-O-Methylasterric acid, Dimethyl 2,30-Dimethylosoate, Questin, (+)-20**S**-Isorhodoptilometrin, 6-hydroxy-8-methoxy-3-Methylisocoumarin, and Trypacidin	Solid state fermentation	**ethyl acetate**
Hussein et al (2022)	Cyclo-(Leu-Pro), Cyclo-(Phe-Pro), Isosclerone, 9-Deacetylfumigaclavine C, Cyclotryprostatin A, Fumigaclavine B, Fumigatoside F, Pseurotin A, Spirotryprostatin A, Hexylitaconic Acid, Fumigaclavine C, 6-Methoxyspirotryprostatin B, Tryptoquivaline F, Chaetominine, 9-Deacetoxyfumigaclavine C, Synerazol, Azaspirofurans B, Monomethylsulochrin-4-sulphate, Fumagiringillin, Questin, Fumitremorgin B, Pyripyropene A, Azaspirofurans A, Monomethyl sulochrin, Methylorsilinate, Fumitremorgin C, Ethylα-D glucopyranoside ,Emodin, 6,16-O-Dideacetyl helvolic acid 21,16-lactone, 16-O-Deacetylhelvolic acid 21,16-lactone, Helvolic acid, 16-O-propionyl-16-Odeacetylhelvolic acid/6-O-propionyl-6-Odeacetylhelvolic acid, 6-O-propionyl-6,16-Odideacetylhelvolic acid21,16-lactone, Pyripyropene F, Pyripyropene O, Linoleic acid, Oleic acid, 5,8-Epidioxyergosta6,9(11),22-trien-3-ol, Ergosterol peroxide, (22E)-Ergosta4,6,8(14), 22,24(28)-pentaen-3-one, Ergosta4,6,8(14),22-tetraen3-one, Ergosterol.	Liquid fermentation	**Ethyl acetate**
Harman G. et al., (2023)	N-formyl-4-hydroxyphenyl-acetamide Tartric acid	Liquid fermentation	**Ethyl acetate**

Table 2: Various studies of antimicrobial metabolites of *A fumigatus*

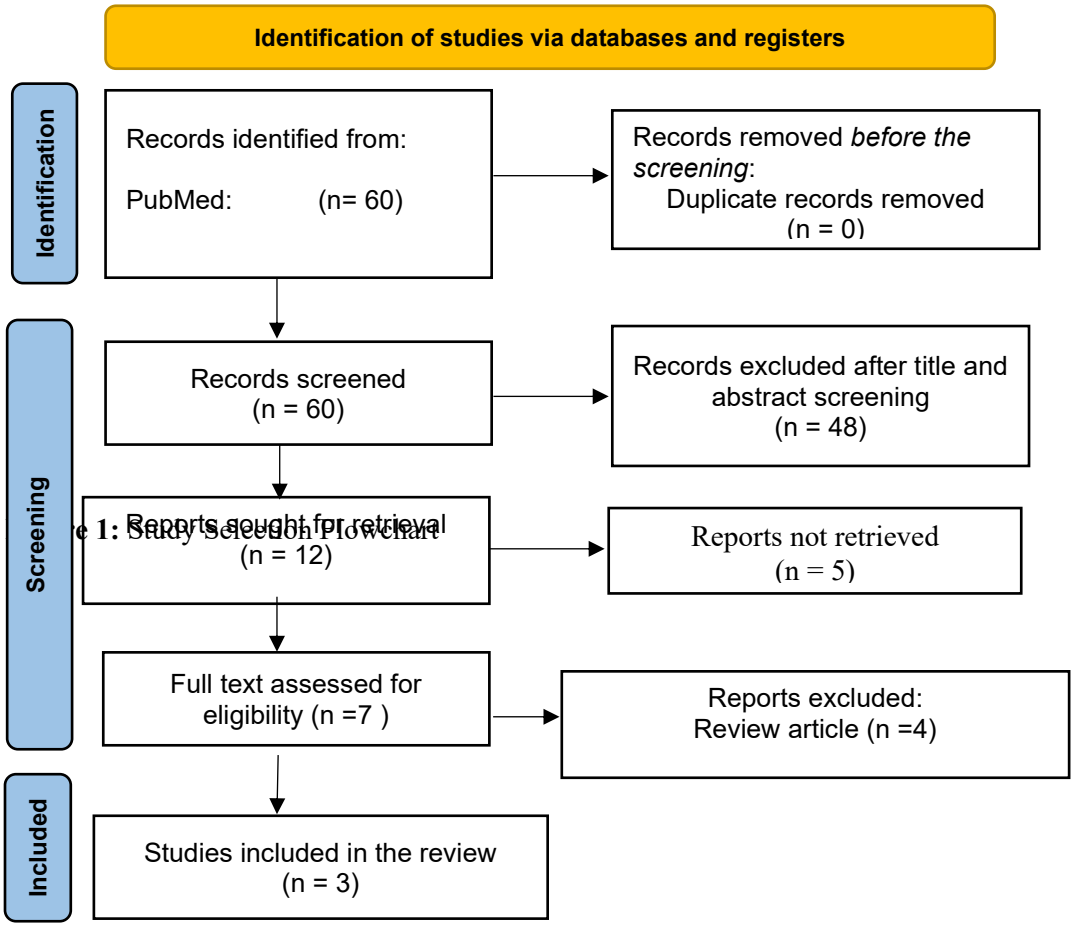
Study	Metabolite produced	Method of metabolite Extraction	Source of *A. fumigatus*	Solvent Used	Organism Tested	Bioassay method	ZoI

Zhang et al. (2022)	Fumindoline A, Fumindoline B, Fumindoline C, 12β, 13β-Hydroxyasperfumigatin, Asperfumigatin, Demethoxyfumitremorgin C, Fumitremorgin C, 1,13-Dihydroxyfumitremorgin C,12*a*-hydroxy-13-Oxofumitremorgin C, Fumitremorgin B, 13-Oxofumitremorgin B, Cyclotryprostatin B, Verruculogen, 6-Methoxyspirotryprostatin B, -Spirotryprostatin A, Spirotryprostatin C, 2-epi-Tryptoquivaline F, Fumiquinazoline C, (+)-Alantrypinone, Oxoglyantrypine, Chaetominine, 11-Epi-Chaetominine, Fumigaclavine C, Bisdethiobis(Methylthio)gliotoxin, Pyripyropene A, Pseurotin F1, Pseurotin F2, Pseurotin A, 11-O-Methylpseurotin A, Azaspirofuran B, Azaspirofuran A, Fumagiringillin, Fumagillin, Helvolic acid, 6-O-propionyl-16-O-deacetylhelvolic acid, 16-O-propionyl-6-O-deacetylhelvolic acid, Penibenzophenone E, Sulochrin, Monomethylsulochrin, 80-O-Methylasterric acid, Dimethyl 2,30-Dimethylosoate, Questin, (+)-20**S**-Isorhodoptilometrin, 6-hydroxy-8-methoxy-3-methylisocoumarin, and Trypacidin.	Solid state fermentation	Sea water of western pacific	Ethyl acetate	MRSA, *Mycobacterium bovis* *Candida albicans.*	Microdilution method	**1.25 to 25 µM** **25 µM** **50 µM**
Hussein et al (2022)	Cyclo-(Leu-Pro), Cyclo-(Phe-Pro), Isosclerone, 9-Deacetylfumigaclavine C, Cyclotryprostatin A, Fumigaclavine B, Fumigatoside F, Pseurotin A, Spirotryprostatin A, Hexylitaconic Acid, Fumigaclavine C, 6-Methoxyspirotryprostatin B, Tryptoquivaline F, Chaetominine, 9-Deacetoxyfumigaclavine C, Synerazol, Azaspirofurans B, Monomethylsulochrin-4-sulphate, Fumagiringillin, Questin, Fumitremorgin B, Pyripyropene A, Azaspirofurans A, Monomethyl sulochrin, Methylorsilinate, Fumitremorgin C, Ethylα-D glucopyranoside, Emodin, 6,16-O-Dideacetyl helvolic acid 21,16-lactone, 16-O-Deacetylhelvolic acid 21,16-lactone, Helvolic acid, 16-O-propionyl-16-Odeacetylhelvolic acid/6-O-propionyl-6-Odeacetylhelvolic acid, 6-O-propionyl-6,16-Odideacetylhelvolic acid21,16-lactone, Pyripyropene F, Pyripyropene O, Linoleic acid, Oleic acid, 5,8-Epidioxyergosta6,9(11),22-trien-3-ol,	Liquid fermentation	Plant leaves (Albizia lucidior)	Ethyl acetate	*S. aureus*	Microdilution method and agar well diffusion	**3.90 µg/mL**

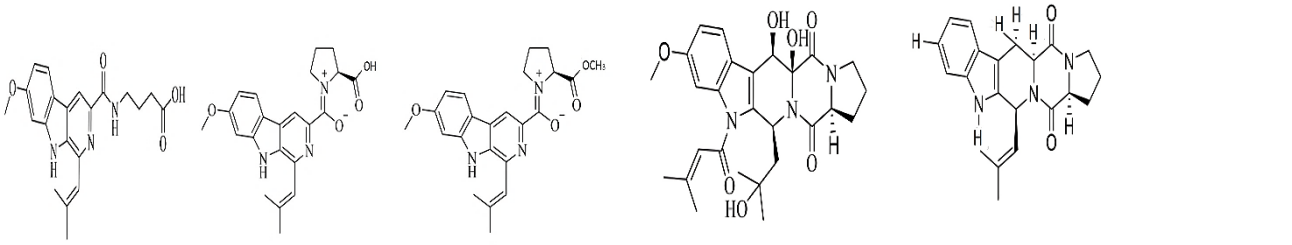
	Ergosterol peroxide, (22E)-Ergosta4,6,8(14), 22,24(28)-pentaen-3-one, Ergosta4,6,8(14),22-tetraen3-one, Ergosterol.						**1.95 µg/mL** **15.63 µg/mL**
Harman G. et al., (2023)	N-formyl-4-hydroxyphenyl-acetamide, Atraric acid.	Liquid fermentation	Soil	Ethyl acetate	*Acinetobacter baumanii*	agar well diffusion method	**19.1 mm** **10.2 mm**

Table 3 Comparison of *A. fumigatus* Metabolites with Existing Antibiotics

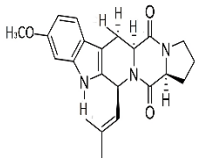
Feature	***A. fumigatus* Metabolites**	**Conventional Antibiotics**
Mechanism of Action	Often novel (e.g., protein synthesis inhibition, redox cycling, MetAP2 inhibition)	Known mechanisms (e.g., β-lactams inhibit cell wall synthesis, aminoglycosides target ribosomes)
Spectrum of Activity	Mostly narrow, some broad (e.g., gliotoxin active against Gram-positive bacteria, and fungi)	Varies from narrow (penicillin) to broad-spectrum (tetracycline, ciprofloxacin)
Potency	High in vitro activity (e.g., gliotoxin is very potent), but can be cytotoxic	Optimized for therapeutic use with better safety profiles
Toxicity	Many are cytotoxic or immunosuppressive (e.g., gliotoxin is toxic to mammalian cells)	Typically, lower toxicity due to extensive optimization
Resistance Potential	Less studied, but some have novel targets = lower pre-existing resistance	Many faces widespread resistance due to overuse (e.g., MRSA, ESBL)
Drug Development Stage	Mostly at the discovery or preclinical stage	Clinically approved and regulated



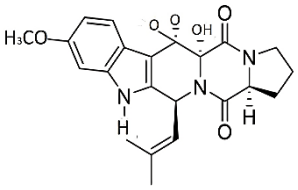
e 1: Study Selection Flowchart



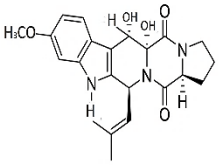
Fumidoline A Fumidoline B Fumidoline C Asperfumigatin Demethoxyfumitremorgin C



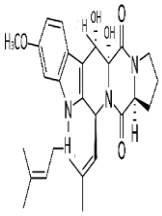
Fumitremorgin C



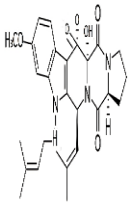
12,13Dihydroxyfumitremorgin C



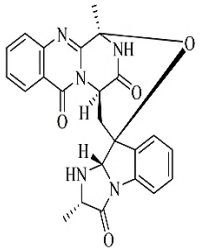
12a-Dihydroxofumitremorgen C



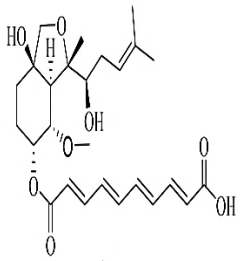
Fumitremorgin B



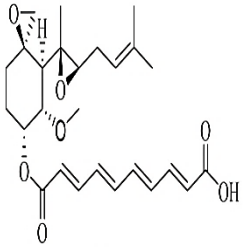
13-oxofumitremorgin B



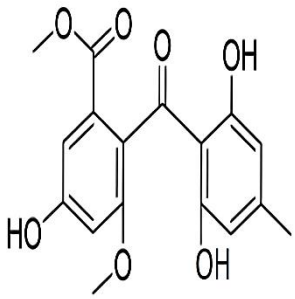
Fumiquinazoline C



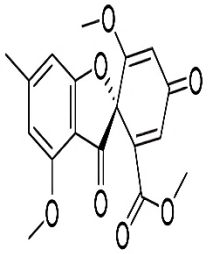
Fumagiringillin



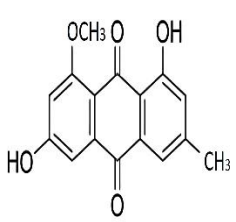
Fumagillin



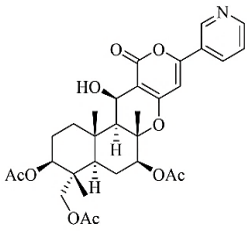
Solorochin



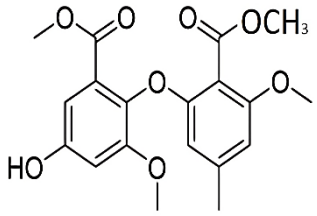
Trypacidin



Questin

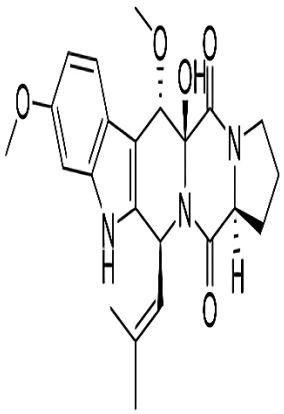


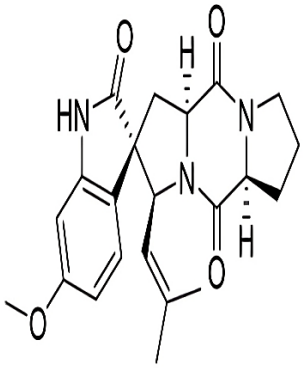
Pyripyropene A



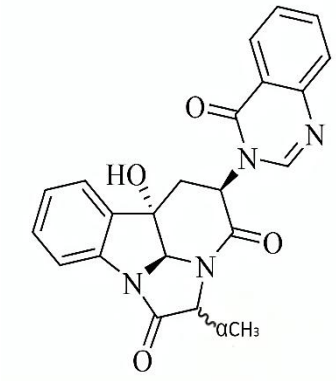
Dimethyl 2,30-dimethylosoate

Figure 2: Selected Polyketides isolated from *A fumigatus*

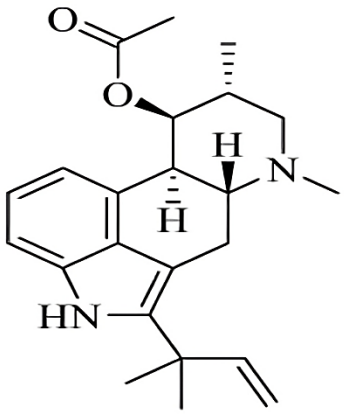


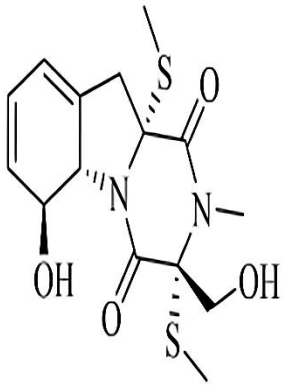


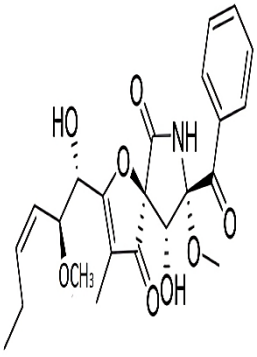
Spirotryprostatin A



11-epi-chaetominine



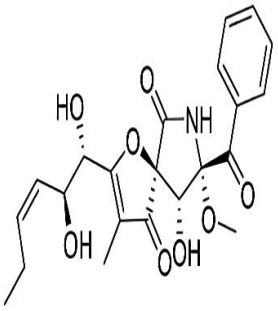


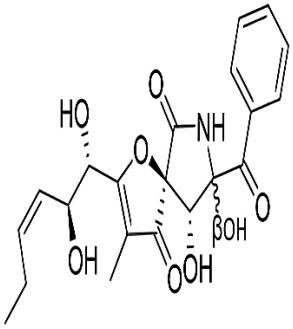


11-O-methylpseurotin A

Fumigaclavine C

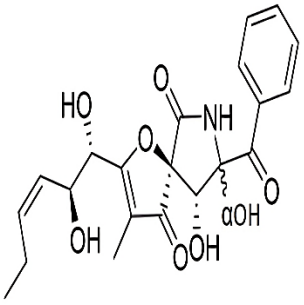
Bisdethiobis(methylthio)gliotoxin



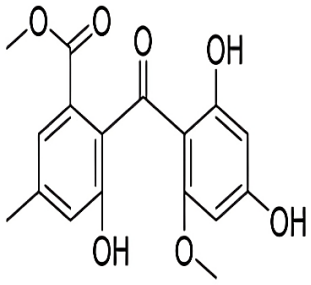


Pseurotin A

Pseurotin F1

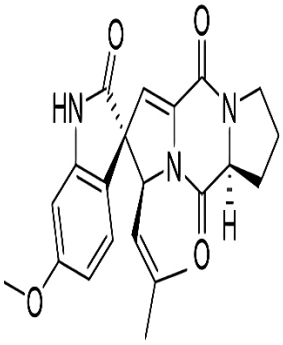


Pseurotin F2

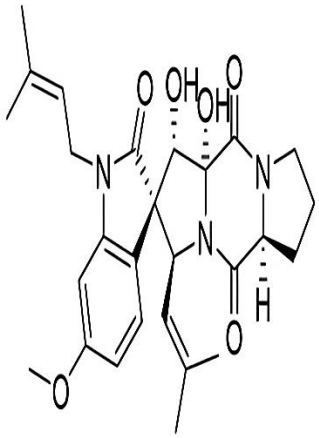


Penibenzophenone E

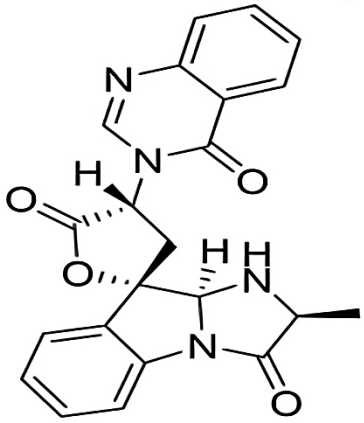
Figure 3: Selected Peptides isolated from *A fumigatus*



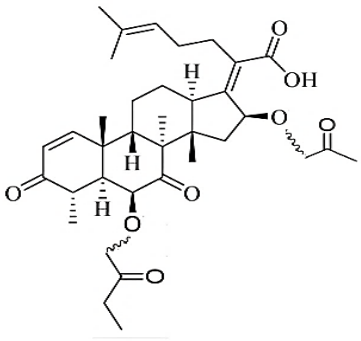
6-methoxyspirotryprostatin B



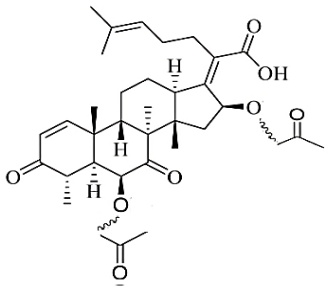
Spirotryprostatin C



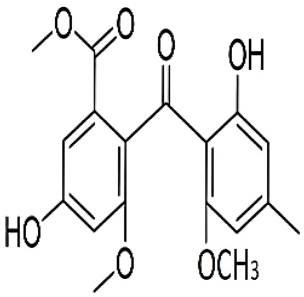
2-epi-tryptoquivaline F



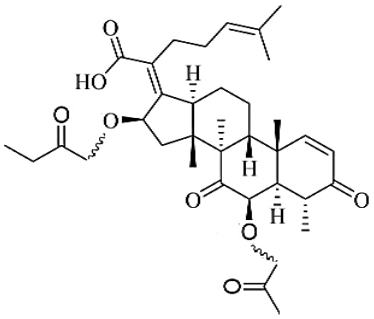
Helvolic acid



6-O-propionyl-16-O-deacetylhelvolic acid



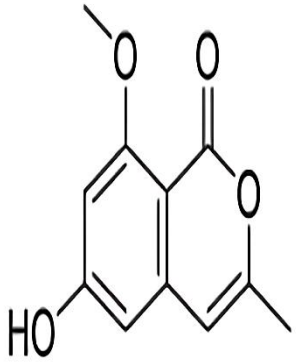
16-O-propionyl-6-O-deacetylhelvolic acid



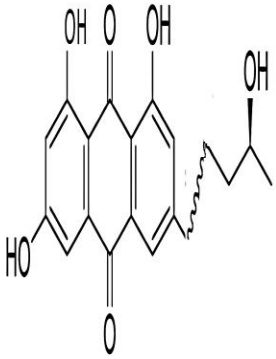
Monomethylsulochrin

6-hydroxy-8-methoxy-3-methylisocoumarin

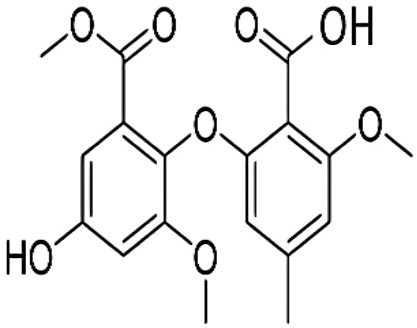
(+)-20S-isorhodoptilometrin

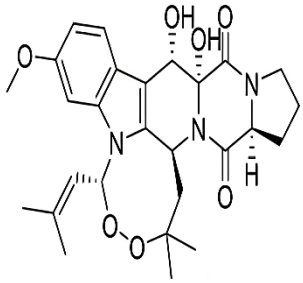


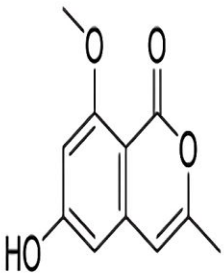
80-O-methylasterric acid



Verruculogen,

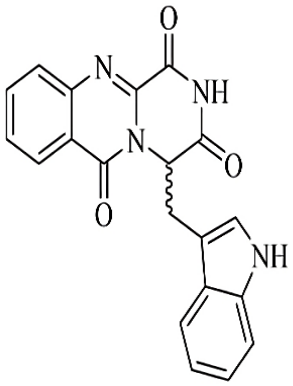




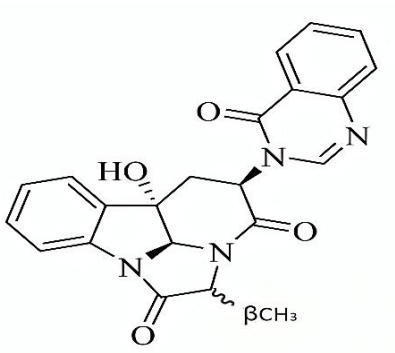


6-hydroxy-8-methoxy-3-methylisocoumarin

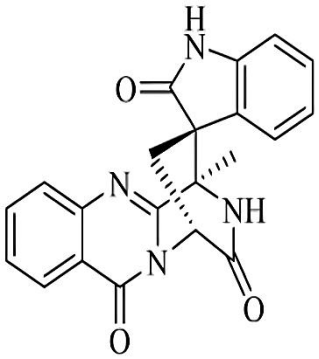
Figure 4: Selected Terpenoids isolated from *A fumigatus*



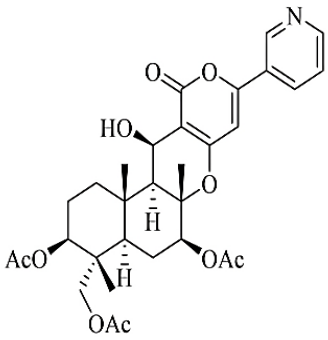
Oxoglyantrypine



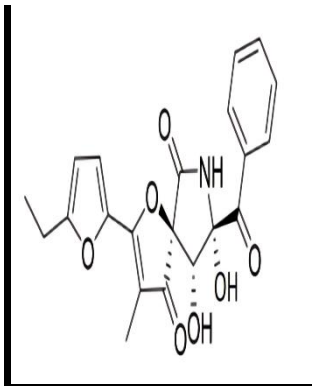
Chaetominine



(+)-alantrypinone, Oxoglyantrypine



Azaspirofuran A



Azaspirofuran B

Figure 5: Selected Terpenoids isolated from *A fumigatus*