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Review Article

Aspochalasins, a Structurally Diverse Fungal Derived Bioactive Sub-group of Cytochalasans and Their Activities

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ABSTRACT

Aspochalasins are a structurally diverse subgroup of cytochalasans produced by fungal secondary metabolism. While much is still unknown about their precise mechanisms of action, they appear to elicit cytostatic and cytotoxic effects similarly as their parental group. Those effects include antibacterial, anti-tumoral, phytotoxic, anti-proliferative, and anti-viral activities. Their potencies are often as varying as their structures with minor modifications on their frequently substituted macrocyclic scaffold causing complete loss or significant enhancement of activity. Due to their various biomedically relevant effects and a large number of macrocyclic carbons with tolerance for diverse functional group substitutions, aspochalasins appear to be good candidates for structural optimization which may produce compounds with potent and targeted effects. This article aims to categorize the underline features of this subgroupof naturally produced compounds.

Keywords: Aspochalasins; Cytochalasans; *Aspergillus*; Fungal metabolites

INTRODUCTION

Cytochalasans are a large class of fungal secondary metabolites with varying anti-tumoral, anti-microbial, and other biological activities [1]. Since their first discovery in 1967, the size of this class has steadily grown and is now made up of at least 300 compounds [2], which are characterized by a highly substituted isoindole ring fused to a larger often 11 or 12 membered macrocyclic ring (Figure 1).

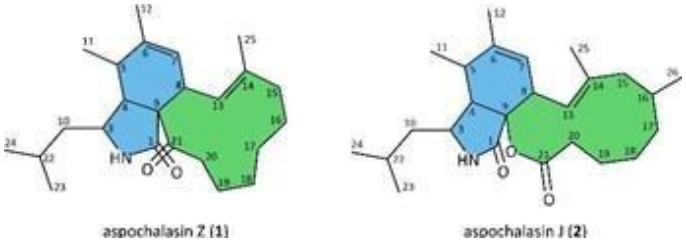

Figure 1: Representative planar structures of aspochalasin Z (1)with a 11-membered macrocyclic ring and aspochalasin J (2) with a 12-membered macrocyclic ring. Isoindole unit is drawn in blue and macrocycle in green.

Cytochalasans with a 2-methylpropyl substitution at C-3 of the isoindole unit are sub-grouped as aspochalasins (Figure 1). By 2004 only 12 such compounds containing this substitution had been identified [3]. As of April 2021, this number has swelled togreater than 100; the associated online Supporting Information provides a compilation of names, structures and sources of thosenatural products, and thus serves as a reference for compounds whose structures are not presented in this article. A majorityof aspochalasins have been isolated as white or pale-yellow amorphous powder from *Aspergillus* species; however, they have also been found to be produced by other fungal species such as Phoma, Periconia, Spicaria, Trichoderma or Westerdykella [4- 18]. This small but rapidly expanding group of fungal natural products have demonstrated proclivity for vast structural diversity and varying biological activities. This article aims to categorize the underline features of aspochalasins.

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STRUCTURAL DIVERSITY ANDCATEGORIZATION

While aspochalasins are easily characterized and recognizable by their C-3 2-methylpropyl group, the macrocycle affords the most opportunity for structural novelty via addition of functional groups or attachment of large complex molecules on the C-17,
-18, -19, and/or -20 positions.

Commonly, aspochalasins are described based upon their overall ring structure. The most common ring arrangement is the tricyclic 5/6/11 organization as seen in aspochalasin Z 1; (Figure 1); however, other ring arrangements such as 5/6/12 as seen in aspochalasin J 2; (Figure 1), or 5/6/6/7, 5/6/9, 5/6/7 have also been identified.

In addition to the previously mentioned ring structural categorization, aspochalasins can also be classified as ketone aspochalasins for having a key C-21 ketone or ester aspochalasins for having a C-21 ester on the macrocycle fused to C-9 of the isoindole ring (Figure 1), or as open-ring aspochalasins for lack of a macrocyclic ring. The exceptions to this rule are a hydroxyl group seen on C-21 of aspochalazine A attributable to its iconic azabicyclo moiety [19], a replacement of the ester ketone with NH in flavichalasine O [20], and periconiasins D-F due to theirseverely truncated macrocycles [9].

Ketone Aspochalasins

Roughly 2 of every 3 (80/117) aspochalasins identified to date contain what can be considered the basic form of the macrocycle with a C-21 ketone moiety (Figure 2). 1 is thought to be the "basic version" of all aspochalasins with the emblematic C-5, -6, and -14 methyl groups but no further modifications to the macrocycle orthe isoindole ring [21].

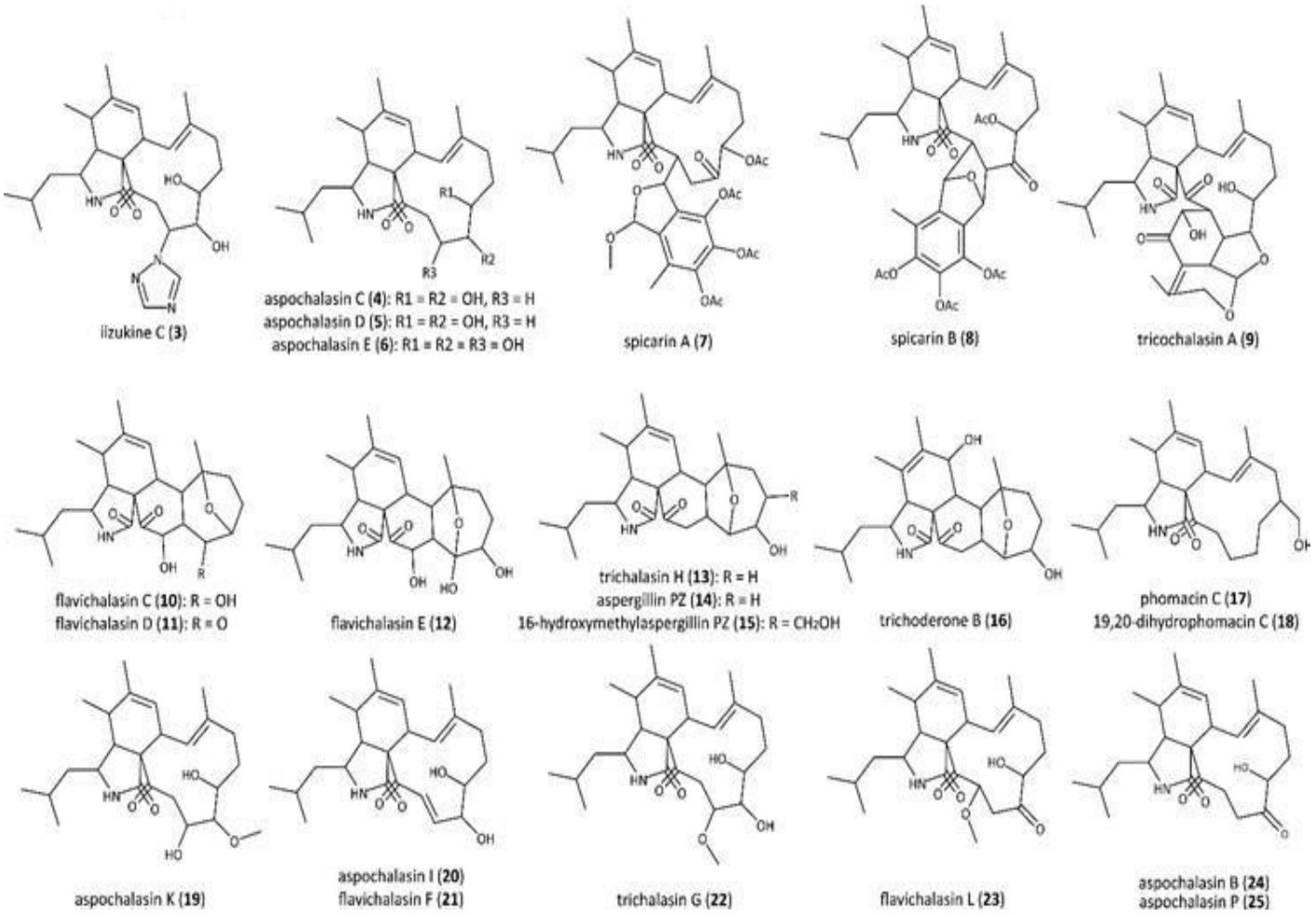

Figure 2: Representative planar structures of the ketone subgroup of aspochalasins.

Though this group contains the most common and basic 5/6/11 ring structure, it nonetheless encompasses interesting compounds such as iizukine C (3) with a unique 1,2,4-triazole group attached to C-19 [22], in addition to the more familiar compounds such as aspochalasins C-E (4-6) with hydroxyl groups at the frequently substituted C-17, -18, and/or -19 positions (Figure 3) [23].Spicarins A-B (7-8) have four acetyloxy groups attached to C-17 and their macrocycle-fused isobenzofuran ring [11]. Tricochalasin A (9) markedly contains a heavily modified 5/6/6 tricyclic ring attached to C-18, -19, and -20 [24].

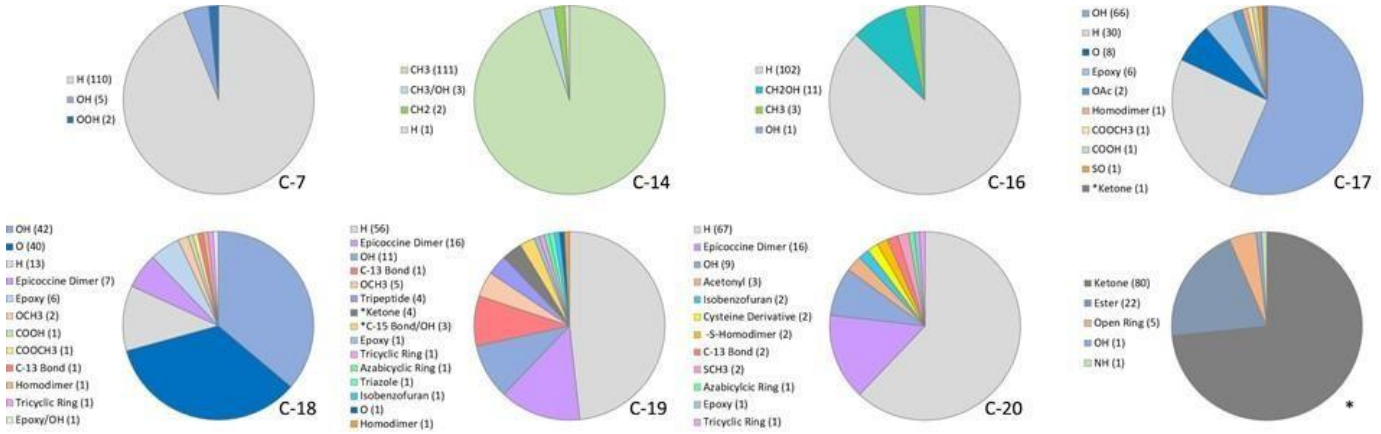

Figure 3: Occurrences of functional attachment to C-7, -14, -16, -17, -18, -19, and -20 of aspochalasins as well as their structural categorization (*) based upon the carbon connecting the macrocycle to the isoindole ring. With few exceptions, aspochalasins typically have a methyl group attached at C-14 and an unsubstituted C-16. For C-17, -18, -19, -21 and -21, substitutions are often oxygenatedfunctional groups or larger high MW modifications.

Despite the presumed availability of C-13 and C-15 on the macrocycle for functional group substitutions, there is no example of substitution on those atoms other than internal ring closure asseen in flavichalasines C-E (10-12), trichalasin H (13), aspergillin PZ (14), 16-hydroxymethylaspergillin PZ (15), and trichoderone B(16). Ring closure often generates an epoxy moiety fused to themacrocyclic ring [16-18,25,26].

Apart from C-13 and C-15, the next most uncommonly substitutedatom in the macrocycle is C-16 (Figure 3). Phomacin C (17) [4,6] and several naturally occurring analogs of 17 have been isolatedand found to have an atypical hydroxymethyl attachment at C-16 including bioactive 17 diastereomer 19,20-dihydrophomacin C (18) [5]. Among these analogs, several were also determined to have an equally rare methoxy group. While methoxy has not yetbeen observed at C-17, it is seen at C-18 in aspochalasin K (19)[3]. Methoxy has also been seen at C-19 of trichalasin G (22) [16],which is an analog of aspochalasin I (20) [3] and flavichalasine F (21) [25]. Finally, a methoxy group is present at C-20 in flavichalasine L (23) [25].

Complex ring systems expand far past the relatively simple tricyclic, tetracyclic, and pentacyclic structures yielding compounds such as the cage-like hendecacyclic epicochalasines A-B, which are thought to be generated from the fusion of an epicoccine dimer to 5 [27] similar to asperflavipines A-B, which are characterized by their tetradecacylic and nonacyclic structures, respectively [2]. Epicoccine is likewise integrated into the structure of asperchalasines A-H which theoretically arise from aspochalasins B and P (24-25) and aspergilasines A-D from 5 [28-31].

Multifarious ring structures are not the only characteristics which give aspochalasins their rich diversity; many identified aspochalasins have interesting groups attached to their macrocycle rather than complex interconnections. Aspochalamins A-D is notable for tripeptide sequences attached to the macrocycle of 1or 5 and were only found in stationary culture [21,32].

Within the ketone aspochalasin group are other oddities such as bisaspochalasin A wherein a 5 monomer is homodimerically linked at C-17, -18, and -19 to the cleaved macrocycle ofanother [33]. In the same way, bisaspochalasins B and C are homodimerized 24 but are instead linked by a peculiar thioether bridge and differentiated by bisaspochalasin C's equally odd C-7 peroxy seen only one other time in trichalasin E [16,33]. While sulfur is rarer than carbon, nitrogen and oxygen in microbial natural products methylthio containing aspochalasins V and W [34] as well as modified cysteine containing cyschalasins A and B [35] have been described. Periconiasin H rounds out the sulfur-containing aspochalasins, however, its sulfoxide group is not its most striking structural feature. The periconiasins are much smaller than typical aspochalasins with ring structures as small as 5/6/7 seen in periconiasin G [8]. Periconiasins D-F are technically not ester nor ketone aspochalasins due to a C-15/18 ring closure on their truncated macrocycle [7,9]. Regardless, the periconiasins still retain the other key aspochalasin characteristics such as the C-3 2-methylpropyl group and isoindole subunit [7-9].

The last common modification is addition of a hydroxyl group to C-7 of the isoindole ring, as seen in 16, aspochalasins L, U and W [36,37], and trichalasin C [15]. This group of compoundsrepresent the lone non-macrocyclic aspochalasin modifications thus far documented (Figure 3).

Ester Aspochalasins

Ester aspochalasins have their macrocycle connected to the isoindole unit by what is now known to be a Baeyer-Villiger generated C-21 ester [38,39] and are largely 5/6/12 structured compounds, though unsurprisingly this is not the only possible ring structure (Figure 4). Aspochalasin F (26) was the first documented occurrence of a C-21 ester aspochalasin reported in 1997 [40]. This group is no less diverse than the ketone aspochalasins and often contains compounds which share structural similarity with analogous ketone counterparts. For example, the uncommon 17, 18 epoxy group found in C-21- ketone aspochalasin G is also seen in the ester-containing 26 [40]. Likewise, many ester aspochalasins are also present with hydroxyl or carbonyl groups attached to C-17, -18, and/or -19 (Figure 3)

[3,22,25,38,41] with internal ring structures such as those seen in amiaspochalasins B and C (27-28) [38].

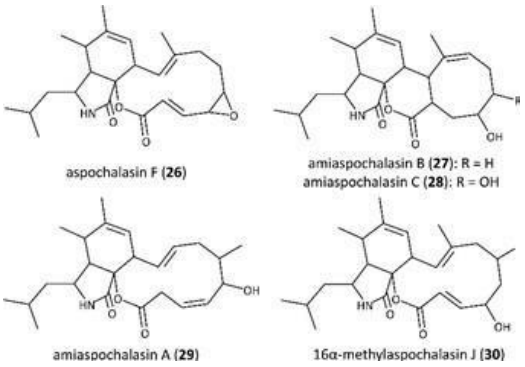

Figure 4: Representative planar structures of the ester subgroup of aspochalasins.

Exceedingly unique amiaspochalasin A (29) has a methyl groupaffixed to C-16 rather than the largely ubiquitous 14-methyl placement seen in nearly all of its aspochalasin congeners (Figure 3) [38]. Similar absence of 14-methyl is only seen in periconiasin F and flavichalasine A [25] which both contain 14-methylene making 29 the only recognized C-14 unsubstituted aspochalasin. Similar C-16 methylation is seen in 16α-methylaspochalasin J (30)

[18] and phomacin A [41] which both, however, retain 14-methyl; interestingly, none of these 16-methyl bearing compounds were isolated from *Aspergillus* sp.

Open-ring Aspochalas

Opening of the macrocycle rather than a ring closure is the source of the last major category of aspochalasins (Figure 5) [42]. The products of those ring breakages have been from both ketone and ester aspochalasins and can occur at many locations on the macrocycle. For example, amiaspochalasin F and G (31-32) [38] are thought to be generated by cleavage of the C-21 ester group from their immediate precursors. Similar ring opening is seen in amiaspochalasin I [42], secochalasins A and B (33-34) [35], and a monomer of the strangely cross-linked bisaspochalasin A [33]. Overall, the open-ring aspochalasins are a small and rare subsetof aspochalasins (Figure 3).

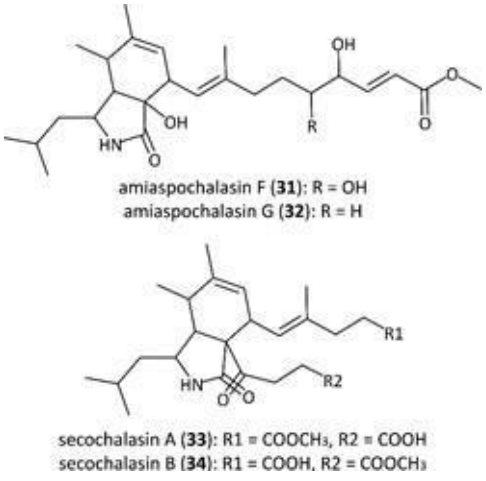

Figure 5: Representative planar structures of the open-ring subgroup of aspochalasins.

Whether those are true terminal secondary metabolites, shunt intermediates during biosynthesis or simply breakdown productsfrom the isolation process is unknown as they have not been reported to have meaningful bioactivities.

BIOSYNTHESIS OF ASPOCHALASINS

While the biosynthesis of aspochalasins is not as thoroughly documented as cytochalasans, limited studies demonstrate that their formation is similar to the parental cytochalasan class which arises from hybrid polyketide synthase–nonribosomal peptide synthase (PKS–NRPS) biosynthetic pathways [43,44]. As aspochalasins are solely distinguished from cytochalasans by their characteristic C-3 2-methylpropyl group, it was postulated that their origins are due to incorporation of leucine in the na-scent structure rather than tryptophan, phenylalanine, tyrosine,valine, or alanine [24,43] which gives rise to other well-known cytochalasan subgroups such as the chaetoglobosins, pyrichala- sins, and alachalasins [35,43].

Specifically, a 36,705-bp biosynthetic gene cluster, ffs, was iden-tified in marine-derived A. flavipes CNL-338 that yields many aspochalasins including 4, 6, 14, 21, aspochalasin M, TMC-169, and flavichalasine G [44]. The ffsA PKS module iteratively in- corporates malonyl-CoA building blocks followed by addition ofL-leucine by an NRPS module [45].

Similarly, a PKS-NRPS gene cluster phm was upregulated by overexpression of transcriptional regulatory gene phmR in Parastagonospora nodorum, which resulted in the production of a previously characterized phomacin derivative [5] that was renamed phomacin D (35) and phomacins E-F 36-37; (Figure 6) [46]. Further, when phmA and phmE were expressed in A. nidulans, prephomacin (38) was produced. While 38 is not a conventional aspochalasin, it was proposed that this compound is the precursor to 35, which can logically be derived following enolization and a Diels-Alder addition [46].

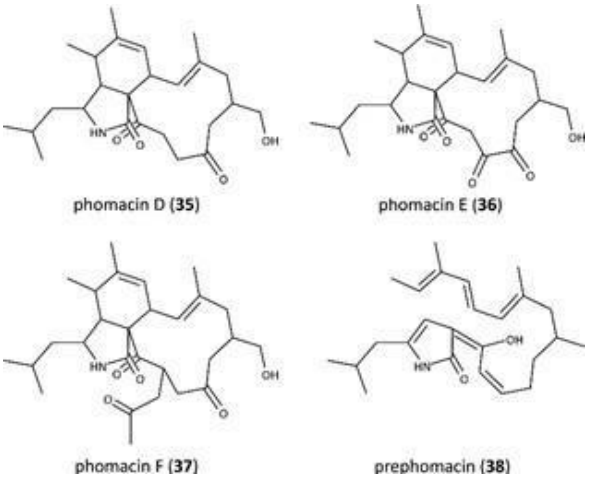

Figure 6: Planar structures of phomacins D-F and prephomacin. 37 notably has a C-20 acetonyl group [46], which is similarly

seen in aspochalasins N and O [13]. Whether this functional group is naturally occurring is unclear; though they appear to bean artifact of using acetone during extraction processes [13,46].Experiments to verify this theory have emphasized the reactivity of C-20 [46].

Synthetically generating those often complex molecules from their biologic precursors complements the ability to understandtheir biosynthetic origin. This has proven to be an achievable endeavour even in the intricate asperchalasine structures [28,29]to aid in understanding their ability to selectively inhibit cell cycling [47].

The proposed biosynthetic pathway for the unique sulfur- containing aspochalasins involves a 1,4-addition of cysteine to intermediate 24 followed by either a deamination-methylationor acylation decarboxylation step to arrive at the structure of cyschalasins A and B, respectively [35]. A similar biosynthetic strategy is proposed for the sulfur-containing bisaspochalasins Band C wherein cysteine is also postulated to conduct a nucleo-philic addition to the unsaturated C-20 of 24 [33].

The complexly linked epicochalasines A and B are suggested to be formed through a Diels-Alder reaction of 5 and two epicoc-cine molecules followed by (3+2) cycloaddition and a subse- quent nucleophilic addition step to yield epicochalasines A or Bdepending on the original orientation of epicoccine at the timeof cycloaddition [27]. A similar biogenetic pathway is proposed for the creation of aspergilasine A which undergoes an intramo- lecular (3+2) cycloaddition to arrive at its final structure [31].

Biological Activities

As indicated by the Greek root meaning 'cell relaxation' [48], cytochalasans are known to elicit anti-proliferative activity through targeting and capping the actin cytoskeleton [49]. This actin capping affects normal cell processes such as cell adhesion, motility, signalling, and cytokinesis [1,35]. Though the biological activity of cytochalasans is well documented, aspochalasins have sporadically reported data (Table 1) which obfuscates our ability to determine their importance, structure-activity relationship, and any specific mechanisms of action which vary from current understanding of cytochalasan activity. For example, cytochalasans have been shown to elicit cytotoxic as well as cytostatic effects, which would presumably allow for the use of the cytostatic compounds in cancer therapy despite their lack of cytotoxicity [43]; whether aspochalasins operate similarly is not extensively studied. Importantly, there is a need for some levels of modification on the macrocycle to cause cytotoxicity as the aspochalasin 'basic version', 1, has no documented bioactivities[21].

Table 1: Reported bioactivities of aspochalasins against cancerousor normal cell lines or microorganisms.

Compound	Notable Inhibitory Bioactivities
aspochalasin C (4)	B16-F10, HCT-116, NCI-H460, MCF-7, SF-268
aspochalasin D (5)	A. globiformis, A. aurescens, A. oxy- dans, A. pascens, B. [3, 15, subtilis, R. erythropolis, S. aureus, S. epidermid- is, Ba/F3, 21, 24, NCI-H460, MCF-7, SF-268, HeLa, PC3 41, 50]
aspochalasin E (6)	B16-F10, HCT-116, NCI-H460, MCF-7, PC3,
	Jurkat, HL60, NB4, HEP3B, RKO, Caspase
	activation, PARP degradation
aspochalasin F (26)	HL60, MH60
aspochalasin G	B. subtilis, S. aureus, M. luteus, A. laidlawii, P. oryzae
	HL60, MH60
aspochalasin I (20)	S. epidermidis, S. aureus, NCI-H460, MCF-7, SF-268,
	Melanogenesis inhibition in Mel-Abcells (IC50: 22.4
	μM)
aspochalasin J (2)	MCF-7, SF-268
aspochalasin K (19)	NCI-H460, MCF-7, SF-268
aspochalasin L	Anti-HIV integrase (IC50: 71.7 μM)
aspochalasin U	TNF-α inhibition
aspochalasin V	PC3, HCT-116
amiaspochalasin D	HL60, A549, SW480
amiaspochalasin E	HL60, A549, Hep3B, U87, SW480
asperchalasine A	cyclin A, CDK2, CDK6, F-actin dis-ruption
asperflavipine A	MDA-MB-231, RKO, Hep3B, HCT116, Jurkat, NB4, HL60
aspochalamin A	A. pascens, B. brevis, R. erythropolis, HM02, MCF7
aspochalamin B	R. erythropolis, HM02, MCF7, HepG2, Huh7
aspochalamin C	A. globiformis, R. erythropolis, HM02, MCF7, HepG2, Huh7

aspochalamin D	R. erythropolis
aspergillin PZ (14)	S. epidermidis, K. pneumoniae, P. aeruginosa, HL-60,
	A2780, PC3, LNCaP, DU145, A2058
bisaspochalasin A	Anti-T-cell proliferation (IC50: 15.8μM)
cyschalasin A	S. aureus, MRSA, C. albicans, HL60, Hep3B, MCF-7, SW480
cyschalasin B	MRSA, HL60, A549, Hep3B, MCF-7, SW480
epicochalasine A	Caspase activation, PARP degrada-tion
epicochalasine B	HL60, NB4, Caspase activation, PARP degradation
flavichalasine F (21)	Jurkat, HL60, NB4, Hep3B, HCT-116, RKO,
	Caspase activation, PARP degrada-tion
iizukine C (3)	HL-60, A549
periconiasin A	HCT-8, BGC-823
periconiasin B	HCT-8, BGC-823, Bel-7402
phomacin A	HT-29
phomacin B (17)	MCF-7, HT-29
phomacin C (18)	HT-29
TMC-169	U937, Jurkat, HL-60, WiDr, HCT-116
trichoderone B (16)	HeLa

35-36 inhibited actin polymerization and 37 did not. These findings suggest that aspochalasins block actin polymerization similar to cytochalasans but these activities are not present in the erroneously generated 37. Further supporting the suggestion of actin inhibition as a mechanism of action, 35-36 both exerted antigerminative phytotoxicity with 35 selectively [50] preventing germination of only monocot Avena sativum and not dicots Arabidopsis thaliana or Lepidium sativum [46].

To date, the most potent aspochalasin reported is TMC-169, a rather simple C-18-hydroxyl analog to 1. Despite or possibly dueto its simplicity, it has shown remarkable cytotoxicity [51] against U937, Jurkat, HL-60, WiDr [52], and HCT-116 (IC50: 0.81, 0.2, 0.68, 0.83, 0.78 μg/ml, respectively) [53]. Another minimally substituted compound, 5, caused cell death in dexamethasone inducible ras-dependent Ba/F3-V12 cells (IC50: 1.9 μg/ml), PC3 cells (IC50: 11.14 μg/ml), and exhibits moderate antimicrobial effects against Gram-positive microorganisms [50].

That is not to say that simplicity is a requirement for activity in aspochalasins. Complex compounds such as the tripeptide-linked aspochalamins elicited moderate growth inhibition zones against Gram-positive microorganisms at 1 mg/ml and aspochalamins A-C had GI50 less than 10 μg/ml against HM02, MCF7, HepG2, and Huh7 cancer cells [21]. Though it was cytotoxic to NCI-H460, MCF-7, and SF-268 [3], 20 interestingly demonstrated no cytotoxic effect on Mel-Ab cells at <100 μM; however, 20 inhibited the ability of Mel-Ab to carry out melanogenesis (IC50: 22.4 μM) by blocking tyrosinase activity [51].

Common to many aspochalasins is having a C-17 hydroxyl or C-18 hydroxyl moiety (Figure 2). There is evidence to support the claim that hydroxyl at C-18 on the macrocycle can be necessary for antibacterial activity. The C-18 hydroxyl of aspochalamin C [21,32] was necessary for its activity against Arthrobacter globiformis when compared to the hydroxyl-lacking aspochalamin D. Similar activity against A. globiformis is seen in 5 which alsobears a C-18 hydroxyl. Interestingly, 5 exerted antibiotic activityagainst many Gram-positive species which were not inhibited by aspochalamins A-D, indicating that their C-19 tripeptide linkagehad a negative effect on their antibiotic potencies [21].

When compared for antibiotic activity, 5, 14, and 20 all caused growth inhibition against S. epidermidis and S. aureus; however,this inhibition is greatly diminished in 14 and 20 (MIC: 20 μM vs. 10 μM) [21,41]. Neither 14 nor 20 are inhibitory to E. coli or B. cereus whereas 5 (MIC: 10 μM) is. 24 was not inhibitory to anyof these organisms (MIC: >20 μM) [41]. Given their structural similarities, it is clear that antibacterial potency among simple 17,18-diol compounds is greater in the C-21 ketone variant and this activity is not enhanced by changing C-17 hydroxyl to C-17 carbonyl or C-17 epoxy. Likewise, the C-17,18-diol-19,20-epoxy bearing aspochalasin H, demonstrated no antibiotic activityagainst these targets [41]. Aspochalasin H also did not prompt cytotoxicity against Ba/F3 cells whereas 5 had high potency (IC50: 0.49 μg/ml) against the same target [54], indicating that this observed C-17 hydroxyl preference may carry over to anticancer effects as well.

While there was little difference in the anticancer activity between 26 and aspochalasin G against HL-60 and MH-60, there was a substantial difference in inhibition zones when 30 μg of either was added to cultures of Gram-positive and Gram-negative species. 26 was inactive whereas aspochalasin G was inhibitory to B. subtilis, S. aureus, M. luteus, A. laidlawii, P. oryzae, and L-form bacteria [40]. Given this, it appears that there is a role forC-21 ketone in antibiotic activity.

As for the effects of internal epoxides, 13-15 have shown anticancer and antimicrobial effects [17,18,26]. Other uncommon compounds such as the 1,2,4-triazole-carrying 3 and the sulfur-containing cyschalasins also have documented cytotoxicity againstcancer cells [22,35].

On the isoindole ring, aspochalasins with C-7 hydroxyl also exhibit important biological activities. Aspochalasin U has shown moderate dose-dependent anti-TNFα activity in L929 cells which led to increased cell survivability by blocking necrotic cell death [36]. Aspochalasin L, which similarly contains C-7 hydroxyl, has shown to inhibit the HIV-1 integrase activity but not HIV replication in HuT78 T-cells (IC50: 71.7 μM) [37].

22 is a C-7-H structural analog to aspochalasin L. Whether 22 has similar anti-HIV integrase activity is unknown. Further study of the comparative effects of these two compounds would aid in determining the importance or lack thereof for the uncommon yet often active C-7 hydroxyl aspochalasins. No activity was observed in C-7 hydroxyl, C-20 methylthio-containing aspochalasin V whereas its C-7 unsubstituted analog aspochalasin W was activeagainst PC3 (IC50: 30.4 μM) and HCT-116 (IC50: 39.2 μM) [34].

Further SAR is possible here due to the number of identified aspochalasins with hydroxyl or methoxy groups (Figure 3).

The number of hydroxyl groups on the macrocycle is not necessarily indicative of the degree to which a compound will be cytotoxic. For example, amiaspochalasins D-E both have C-18 hydroxyl but amiaspochalasin D also has a C-17 hydroxyl. Despite being more substituted, amiaspochalasin D was significantly less cytotoxic to HL60, A549, Hep3B, U87, and SW480 than its structural analog [38].

Investigation of the role of C-16 methyl moiety is possible through comparison of 2 and 30. Though they have not been tested against the same targets, both compounds have shown moderate cytotoxicity [3,18]. Amiaspochalasin A lacked activity against a panel of cancer cell lines [38]. This deficiency in activity may have more to do with the absence of the extremely pervasive C-14 methyl rather than the presence of C-16 methyl.

Further, phomacin A demonstrated potent toxicity against HT- 29 cells (IC50: 0.6 μg/mL) but the toxicity is reduced in C-16 methoxy carrying phomacin B (IC50: 1.4 μg/mL) or its C-21 ketone analogue 17 (IC50: 7.4 μg/mL) [6]. Of a group of six

17 analogs which have been described, none demonstrated cytotoxicity (IC50: >50 μM) against HT-29 except for 18 (IC50:

49.09 μM) [5].

Interestingly, the size of aspochalasins does not appear to be determinative of their activities. Compounds as small as a MW of 359.5 and as large as a MW of 976.2 have been found to be biologically active [7,33]. The smallest active aspochalasins, periconiasins A-B, both showed sub-micromolar potency against HCT-8 (IC50: A: 0.9, B: 0.8 μM). Though they differed only by the orientation of their C-17 hydroxyl moiety, they had dissimilar potency against BGC-823 (IC50: A: 2.1, B: 5.1 μM) and periconiasin B was the only compound of the two to be cytotoxic to Bel-7402 (IC50: 9.4 μM) [7]. The largest aspochalasin discovered to date, asperchalasine A, which consists of two 24 monomers attached to a central epicoccine has exhibited ability to selectively arrest the cell cycle at G1 through inhibition of cyclinA, CDK2, and CDK6 in cancerous cells [47]. Other epicoccine- carrying aspochalasins, epicochalasins A-B and asperflavipine A, have shown cancer cell cytotoxicity through the activation of caspase-3 and degradation of PARP [2,27], a process similarly noted in flavichalasine F [25] (Figure 7).

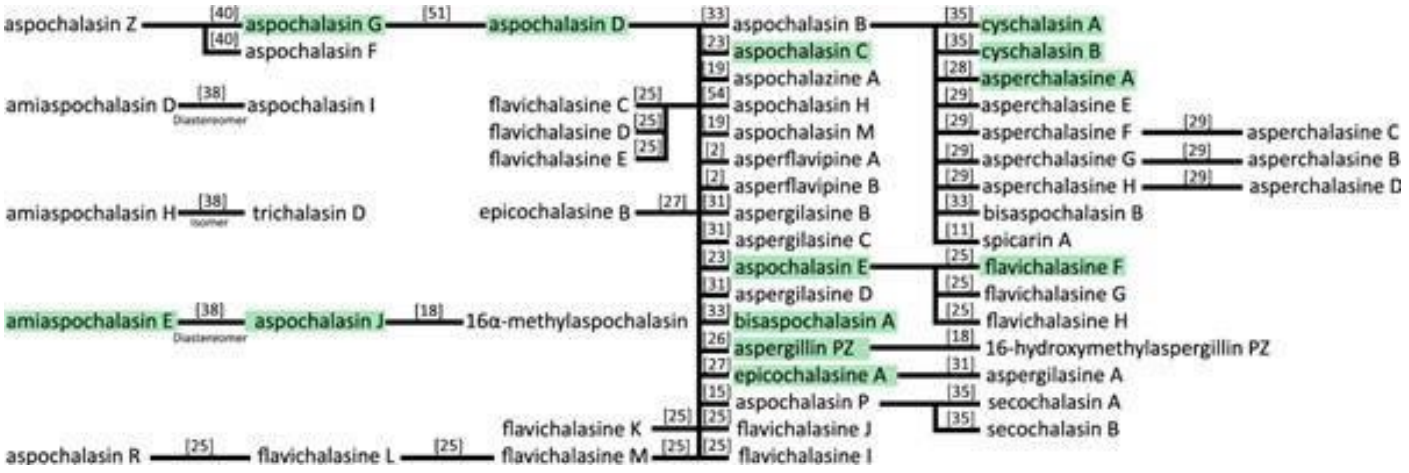

Figure 7: Proposed plausible relationships and biosynthetic derivations of various ketone aspochalasins from the 'basic' aspochalasin Z to more complexly substituted molecules. Compounds highlighted in green have documented biological activity; those n ot highlighted have no documented biological activity though are not necessarily inactive. Numbers in bracket are reference numbers.

CONCLUSION

Much is now known about the vastness of aspochalasin chemical space, though little is currently known about their structure- activity relationships which dictate their prolific anticancer, antimicrobial, phytotoxic and anti-viral profiles. As the size of the aspochalasin subgroup has now eclipsed 100 compounds, there is a necessity as well as an opportunity to delve deeper into theirstructure-activity relationships.

Investigation of the responsible biosynthetic gene clusters may be helpful to discover more cryptic aspochalasins or further understand their biogenesis. Though their structures are often complex, many of these secondary metabolites have analogous compounds which differ by only one functional moiety. Because of this and considering their documented potential to elicit a wide array of biomedically relevant effects, the next sensible step is to methodically analyse the structure-activity relationships against common targets or to attempt synthetic structural optimization particularly on the macrocycle which has shown to permit attachment of substitutions that could potentially increase potency or targeting specificity. Given their well-documented de

novo syntheses and reactive macrocyclic ring, aspochalasins may be appropriate scaffolds for further drug development as it is clear that size does not necessarily preclude aspochalasins from eliciting meaningful activity, though there is some bias for smallermolecules.

Among the potential modifications to the macrocycle, C-14 methyl as well as C-17 and/or C-18 oxygenated functional groups appear to be preferred for bioactivity. 4, 5, and 24 appear to be functional 'starting blocks' for building a large portion of this set of compounds and would thus be ideal candidates for any optimization attempts as their syntheses have been well documented.

Most importantly, the precise mechanism of action of those compounds is still largely unknown. While their ability to cause cytotoxic or cytostatic activity is not disputed and there are suggestions that aspochalasins operate through actin capping, determining whether there are any other mechanistic activities which cause this wide range of intracellular effects is crucial to creating strategies to optimize their structures.

CONFLICT OF INTEREST

There is no conflict of interest to be reported by any of the authors.

REFERENCES

1. Scherlach K, Boettger D, Remme N, Hertweck C. The chemistry and biology of cytochalasans. Natural product reports. 2010;27(6):869-886.
2. Zhu H, Chen C, Tong Q, Yang J, Wei G. Asperflavipine A: A cytochalasan heterotetramer uniquely defined by a highly complex tetradecacyclic ring system from Aspergillus flavipes QCS12. 2017;56(19):5242-5246.
3. Zhou GX, Wijeratne EK, Bigelow D, Pierson LS, VanEtten HD. Aspochalasins I, J, and K: three new cytotoxic cytochalasans of Aspergillus f lavipes from the rhizosphere of Ericameria l aricifolia of the Sonoran Desert. J nat prod. 2004 ;67(3):328-332.
4. Rothweiler W, Tamm C. Isolation and structure of phomin.1966; 22(11):750-752.
5. Xu D, Luo M, Liu F, Wang D, Pang X. Cytochalasan and tyrosine-derived alkaloids from the marine sediment-derived fungus Westerdykella dispersa and their bioactivities. 2017;7(1):1-9.
6. Alvi KA, Nair B, Pu H, Ursino R, Gallo C. Phomacins: Three novel antitumor cytochalasan constituents produced by a Phoma sp. J org chem.1997;62(7):2148-2151.
7. Zhang D, Ge H, Xie D, Chen R, Zou JH. Periconiasins A–C, new cytotoxic cytochalasans with an unprecedented 9/6/5 tricyclic ring system from endophytic fungus Periconia sp.2013;15(7):1674-1677.
8. Zhang D, Tao X, Liu J, Chen R, Zhang M. Periconiasin G, a new cytochalasan with unprecedented 7/6/5 tricyclic ring system from the endophytic fungus Periconia sp.2016;57(7):796- 799.
9. Zhang D, Tao X, Chen R, Liu J, Li L. Pericoannosin A, a polyketide synthase nonribosomal peptide synthetase hybrid metabolite with new carbon skeleton from the endophytic fungus Periconia sp. 2015;17(17):4304- 4307.
10. Zaghouani M, Kunz C, Guédon L, Blanchard F, Nay B. First total synthesis, structure revision, and Natural History of the Smallest Cytochalasin:(+)αPericoniasin G. Chemistry, A Euro J. 2016;22(43):15257-15260.
11. Lin Z, Ma X, Wei H, Li D, Gu Q. Spicarins A D from acetylated extract of fungus Spicaria elegans KLA03. 2015;5(44):35262-35266.
12. Lin ZJ, Zhu TJ, Chen L, Gu QQ. Three new aspochalasin derivatives from the marine-derived fungus Spicaria elegans. 2010 ;21(7):824-826.
13. Lin Z. Spicochalasin A and New Aspochalasins from the Marine- Derived Fungus Spicaria elegans. Euro J Org Chem. 2009;3045-3051.
14. Ding G, Wang H, Li L, Song B, Chen H. Trichodermone, a spiro-cytochalasan with a tetracyclic nucleus (7/5/6/5) skeleton from the plant endophytic fungus Trichoderma gamsii. J nat prod. 2014 ;77(1):164-167.
15. Ding G, Chen L, Chen A, Tian X, Chen X. TrichalasinsC and D from the plant endophytic fungus Trichoderma gamsii. Fitoterapia. 2012;83(3):541-544.
16. Chen L, Liu YT, Song B, Zhang HW, Ding G. Stereochemical determination of new cytochalasans from the plant endophytic fungus Trichoderma gamsii. Fitoterapia. 2014;96:115-122.
17. Ding G, Wang H, Li L, Chen AJ, Chen L. Trichoderones A and B: Two pentacyclic cytochalasans from the plant endophytic fungus Trichoderma gamsii. Euro J Org Chem. 2012 May;2012(13):2516-9.

18. Xu D, Zhang X, Shi X, Xian PJ, Hong L. Two newcytochalasans from the marine sediment-derived fungus Westerdykella dispersa and their antibacterial activities.2019;32:52-55.
19. Li X, Zhao Z, Ding W, Ye B, Wang P.Aspochalazine A, a novel polycyclic aspochalasin from the fungus Aspergillus sp. Z4. 2017;58(25):2405-2408.
20. Si Y, Tang M, Lin S, Chen G, Feng Q. Cytotoxic cytochalasans from Aspergillus flavipes PJ03-11 by OSMAC method.2018;59(18):1767-1771.
21. Gebhardt K, Schimana J, Hoeltzel A, Dettner K, Draeger S et al Aspochalamins AD and Aspochalasin Z Produced by the Endosymbiotic Fungus Aspergillus niveus LU 9575 I. Taxonomy, Fermentation, Isolation and Biological Activities. J antibiotics. 2004 ;57(11):707-714.
22. Kang HH, Zhong MJ, Ma LY, Rong XG, Liu DSet al. Iizukines C– E from a saline soil fungus Aspergillus iizukae. Bioorganic chemistry. 2019;91:103167.
23. Naruse N, Yamamoto H, Murata S, Sawada Y, Fukagawa Y. Aspochalasin E, a new antibiotic isolated from a fungus. J of antibio. 1993 ;46(4):679-681.
24. Li X, Ding W, Wang P, Xu J. Two novel aspochalasins from the gut fungus Aspergillus sp. Z4. Marine drugs. 2018;16(10):343.
25. Wei G, Tan D, Chen C, Tong Q, Li XN. Flavichalasines A–M, cytochalasan alkaloids from Aspergillus flavipes. Scientific reports. 2017; 7(1):1-2.
26. Zhang Y, Wang T, Pei Y, Hua H, Feng B. Aspergillin PZ, a novel isoindole-alkaloid from Aspergillus awamori. The J antibiotics.2002;55(8):693-695.
27. Zhu H, Chen C, Tong Q, Li XN, Yang J. Epicochalasines A and B: Two bioactive merocytochalasans bearing caged epicoccine dimer units from Aspergillus flavipes. Angewandte Chemie International Edition. 2016;55(10):3486-3490.
28. Long X, Ding Y, Deng J. Total synthesis of asperchalasines A, D, E, and H. Angewandte Chemie. 2018 ;130(43):14417-14420.
29. Bao R, Tian C, Zhang H, Wang Z, Dong Z. Total syntheses of asperchalasines A–E. Angewandte Chemie International Edition. 2018;57(43):14216- 14220.
30. KellerαSchierlein W, Kupfer E. Stoffwechselprodukte von Mikroorganismen. 186. Mitteilung. Über die Aspochalasine A, B, C und D.Helvetica Chimica Acta. 1979 ;62(5):1501- 1524.
31. Wei G, Chen C, Tong Q, Huang J, Wang W. Aspergilasines A–D: four merocytochalasans with new carbon skeletons from Aspergillus flavipesQCS12. 2017 ;19(16):4399-4402.
32. Hoeltzel A, Schmid DG, Nicholson GJ, Krastel P, Zeeck A. Aspochalamins AD and Aspochalasin Z Produced by the Endosymbiotic Fungus Aspergillusniveus LU 9575 II. Structure Elucidation. The Journal of antibiotics. 2004;57(11):715-720.
33. Wang L, Yang J, Huang JP, Li J, Luo J. Bisaspochalasins A–C: Three Cytochalasan Homodimers with Highly Fused Ring System from an Endophytic Aspergillus flavipes. Organic Letters. 2020 ;22(20):7930-7935.
34. Liu Y, Zhao S, Ding W, Wang P, Yang X. Methylthio-aspochalasins from a marine-derived fungus Aspergillus sp. Marine drugs. 2014;12(10):5124-5131.
35. Wu Z, Zhang X, Anbari WH, Zhou Q, Zhou P. Cysteine residue containing merocytochalasans and 17, 18-seco-aspochalasins from Aspergillus micronesiensis. J nat prod. 2019;82(9):2653-2658.
36. Liu J, Hu Z, Huang H, Zheng Z, Xu Q. Aspochalasin U, a moderate TNF-αinhibitor from Aspergillus sp. The J of antibio. 2012;65(1):49-52.
37. Rochfort S, Ford J, Ovenden S, San Wan S, George S. Anovel aspochalasin with HIV-1 integrase inhibitory activity from Aspergillus flavipes. The J of antibio. 2005; 58(4):279-283.
38. Wu Z, Zhang X, Al Anbari WH, Zhang M, Chen X. Amiaspochalasins A–H, Undescribed Aspochalasins with a C-21 Ester Carbonyl from Aspergillus micronesiensis. The J org chem. 2019;84(9):5483-5491.
39. Robert JL, Tamm C. Biosynthesis of cytochalasans. Part 5. The incorporation of deoxaphomin into cytochalasin B (phomin). Helvetica chimica acta. 1975 ;58(8):2501-2504.

40. Fang F, Ui H, Shiomi K, Masuma R, Yamaguchi Y. Two new components of the aspochalasinsproduced by Aspergillm sp. The J of antibio. 1997 ;50(11):919-925.

41. Zheng CJ, Shao CL, Wu LY, Chen M, Wang KL. Bioactive phenylalanine derivatives and cytochalasins from the soft coral-derived fungus, Aspergillus elegans. Marine drugs. 2013;11(6):2054-2068.

42. Wang C, Wu X, Bai H, Zaman KA, Hou S. Antibacterial and NF-αB Inhibitory Lumazine Peptides, Aspochalasin, α-Butyrolactone Derivatives, and Cyclic Peptides from a Hawaiian Aspergillus flavipes. J of nat prod. 2020 ;83(7):2233- 2240.

43. Skellam E. The biosynthesis of cytochalasans.Natural product reports. 2017;34(11):1252-1263.

44. Fujii R, Minami A, Gomi K, Oikawa H. Biosynthetic assembly of cytochalasin backbone. 2013;54(23):2999-3002.

45. Heard SC, Wu G, Winter JM. Discovery and characterization of a cytochalasan biosynthetic cluster from the marine-derived fungus Aspergillus flavipes CNL-338. The Journal of Antibiotics. 2020;73(11):803-807.

46. Li H, Wei H, Hu J, Lacey E, Sobolev AN. Genomics-driven discovery of phytotoxic cytochalasans involved in the virulence of thewheat pathogen Parastagonospora nodorum. ACSchemical biology. 2019 ;15(1):226-233.

47. Zhu H, Chen C, Xue Y, Tong Q, Li XN. Asperchalasine A, a cytochalasan dimer with an unprecedented decacyclic ring system, from Aspergillus flavipes. 2015;54(45):13374-13378.

48. Aldridge DC, Armstrong JJ, Speake RN, Turner WB. The cytochalasins, a new class of biologically active mould metabolites.1967;26-27.

Lin DC, Tobin KD, Grumet M, Lin S. Cytochalasins inhibit nuclei-induced actin polymerization by blocking filament elongation.The J of cell bio. 1980 ;84(2):455-460.

49. Tomikawa T, Shin-Ya K, Kinoshita T, Miyajima A, Seto H. Selective cytotoxicity and stereochemistry of aspochalasin D. The J of antibio. 2001;54(4):379-381.

50. Choo SJ, Yun BS, Ryoo IJ, Kim YH, Bae KH. Aspochalasin I, a melanogenesis inhibitor from Aspergillus sp. Journal of microbiology and biotechnology. 2009;19(4):368-371.

51. Erden Y, Tekin S, CEYLAN KB, Tekin C, Kirbag S. Antioxidant, antimicrobial and anticancer activities of the aspergillin pz and terphenyllin secondary metabolites: an in vitro study. Gazi University Journal of Science. 2019 ;32(3):792-800.

52. Kohno J, Nonaka N, Nishio M, Ohnuki T, Kawano K et al.TMC-169, a new antibiotic of the aspochalasin group produced by Aspergillus flavipes. The Journal of antibiotics. 1999 ;52(6):575-577.

53. Tomikawa T, Shin-Ya K, Seto H, Okusa N, Kajiura T et al.Structure of aspochalasin H, a new member of the aspochalasin family. The Journal of antibiotics. 2002 ;55(7):666-668.