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REVIEW ARTICLE

Styrylpyrone-class compounds from medicinal fungi *Phellinus* and *Inonotus* spp., and their medicinal importance

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Members of the genera *Phellinus* and *Inonotus*, including *P. linteus*, *P. igniarius*, *P. ribis*, *I. obliquus* and *I. xeranticus* are well-known medicinal fungi (mushrooms) and have been used in treatment of cancer, diabetes, bacterial and viral infections and ulcer. Adverse effects of these medicinal mushrooms have not yet been reported, indicating the safe nature of these mushrooms. Polysaccharides, particularly β-glucan, are considered the compounds responsible for the biological activity of medicinal mushrooms. However, there is only a limited amount of evidence to indicate that polysaccharides are in fact responsible for the biological effects of these medicinal mushrooms. Recently, many research groups have begun identification of active low-MW compounds in medicinal mushrooms, with a focus on the yellow polyphenol pigments, which are composed of a styrylpyrone class of compounds. Interestingly, a representative group of medicinal fungi, including *P. linteus*, *P. igniarius*, *P. ribis*, *I. obliquus* and *I. xeranticus* were shown to produce a large and diverse range of styrylpyrone-type polyphenol pigments that exhibited various biological activities, including anti-oxidative, anti-inflammatory, cytotoxic, anti-platelet aggregation, anti-diabetic, anti-dementia and anti-viral effects. Styrylpyrone pigments in mushrooms are thought to have a role similar to that of flavonoids in plants. The unique and unprecedented carbon skeleton of fused styrylpyrone might be an attractive molecular scaffold for pharmacological applications. In this review, the structural diversity, biological effects and biogenesis of styrylpyrone-class polyphenols from medicinal fungi are described.
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INTRODUCTION

Mushrooms are ubiquitous in nature and are a good source of food with high nutritional attributes. Some mushrooms have been shown to be physiologically beneficial to humans and to produce various classes of structurally unique and biologically active metabolites that exhibit significant anti-microbial, anti-tumor and anti-viral activities.[1–5] Members of the genera *Phellinus* and *Inonotus*, including *P. linteus*, *P. ribis*, *P. igniarius*, *I. obliquus*, and *I. xeranticus*, have been used as traditional medicines for treatment of gastrointestinal cancer, cardiovascular disease, tuberculosis, liver or heart disease, fester, bellyache, blood gonorrhea, stomach ailment and diabetes.[6] However, despite increased usage, their pharmacological actions have not been well established. Polysaccharides, particularly β-glucan, are believed to be responsible for the biological activity of medicinal mushrooms, and a number of polysaccharides and protein-bound polysaccharides have been used clinically for treatment of cancer. Examples include the polysaccharopeptide Krestin from *Coriolus versicolor*,[7] Mesima from *P. linteus*,[8] Lentinan from *Lentinus edodes*,[9] and Schizophylan from *Schizophyllum commune*.[10] These polysaccharides have been developed and used extensively as anti-cancer drugs in Asia. Although an overwhelming number of reports have been published on the importance of polysaccharides as immunomodulating agents, not all of the curative properties found in these medicinal fungi could be fully accounted for.[11–15]

Recently, these medicinal fungi have been reported to produce a variety of yellow polyphenol pigments, known as styrylpyrones, which have shown significant biological effects, such as anti-oxidative, anti-cancer, anti-platelet, anti-diabetic, anti-inflammatory and anti-viral activities. Since the first isolation of hispidin (**1**) as a naturally occurring styrylpyrone from *Inonotus hispidus* (formerly *Polyporus hispidus*) in 1889, a number of other styrylpyrone metabolites from *Phellinus* and *Inonotus* have been discovered. Styrylpyrone pigments are common constituents of fungi, mainly those belonging to the Hymenochaetaceae family, including *Phellinus* and *Inonotus*.[16] They also exist in primitive angiosperm families, including Piperaceae, Lauraceae, Annonaceae, Ranuculaceae and Zingiberaceae, where they have an important role in defense against mechanical wounding or microbial attack, and show potent biological activities, including

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anti-cancer and sedative effects.[17,18] Biogenesis of fungal styrylpyrones and their intrinsic role in fungi, like the phenylpropanoid derivatives of plants, which have important ecological and physiological roles, have attracted much attention. Styrylpyrones are regarded as phenylalanine (Phe)-derived fungal metabolites with a variety of functions, including defense, pigmentation and signaling molecules.

Classification of fungal metabolites based on their putative biosynthesis was first reported by Turner and Aldridge.[19] Subsequently, in 1987, Gill and Steglich[20] conducted a comprehensive study of styrylpyrones isolated from fungi belonging to the macromycetes. Gill continued this work, and reported on a variety of fungal pigments from 1986 to 2001.[21–23] They were classified as styrylpyrones according to their biogenesis by Dewick, who introduced styrylpyrone and kavapyrone derived from the plant *Piper methysticum*.[18] Styrylpyrones were not discovered until 30 years after hispidin (**1**) and several of its dimers were isolated in the mid 1970 s.

This review includes a discussion of styrylpyrone metabolites isolated from *Phellinus* and *Inonotus* during the past 10 years, and a presentation on recent progress in isolation, structural diversity, biological activities and biosynthesis of styrylpyrones from these medicinal fungi.

STRUCTURAL DIVERSITY OF STYRYLPYRONES

Hispidin (**1**) and bisnoryangonin (**2**) may be the best-known examples of styrylpyrones. Compound **1** was first isolated from *I. hispidus* by Zopf in 1889, but was first identified as 6-(3′,4′-dihydroxystyryl)-4-hydroxy-2-pyrone by Edwards in 1961 and by Bu'Lock in 1962.[20] Labeling experiments in cultures of *I. hispidus* and *Phaeolus schweinitzii* have demonstrated that the styryl group is derived from Phe via cinnamic acid, *p*-coumaric acid and caffeoyl-CoA pathways, whereas the pyrone ring is derived from acetate.[24–26] In addition, compound **1** was shown to be biosynthesized by hydroxylase, which catalyzes hydroxylation of compound **2**. Compounds **1** and **2** were isolated from fungi belonging to the genera *Gymnopilus*, *Hypholoma* and *Pholiota* in the Agaricales, and genera belonging to the family Hymenochaetaceae in the Aphyllophorales (Table 1). However, bisnoryangonin has been found mainly in the Agaricales, and trace amounts have been identified in *Phellinus* and *Inonotus*. Since hispidin (**1**) was first reported as a PKC (protein kinase C) inhibitor in 1977, it has been commercially available for use in standard experiments associated with PKC.[27] Compound **1** from mycelial cultures of *P. linteus* exhibited anti-oxidative and β-secretase inhibitory activities.[28,29]

Remarkably, various hispidin-classes of metabolites have been biogenerated by dimerization or oligomerization of styrylpyrone building blocks (Figure 1). Hispidin (**1**) and bisnoryangonin (**2**) may polymerize during maturation of fungi and production of fungal lignin. Steglich and coworkers characterized 3,14′-bihispidinyl (**3**) from *P. pomaceus*[30] and hypholomines A (**4**) and B (**5**) and fasciculines A (**6**) and B (**7**) from *Hypholoma fasciculare*.[31] Fiasson reported on the presence of hispidin (**1**) and its two dimers, hypholomine B and 3,14′-bihispidinyl, in *Phellinus* and *Inonotus*.[16] Recently, the structurally intriguing phelligridimer A (**8**) was isolated from *P. igniarius*,[32] and two unprecedented 3,3′-fused bis-styrylpyrones, named squarrosidine (**9**) and pinillidine (**10**), were isolated from *Pholiota squarrosa* and *P. pini*, respectively.[33] Compound **10** has the same structure as 1,1-distyrylpyrylethan, which was previously isolated from the fruiting body of *Phaeolus schweinitzii*.[20] Occurrence of styrylpyrones in different families implies that they may have similar ecological roles, such as scavenging for reactive oxygen species in oxidative stress. Recently, we found that hispidin (**1**) and its dimeric compounds, 3,14′-bihispidinyl (**3**), hypholomine B (**5**) and 1,1-distyrylpyrylethan (**10**), existed in cultured broths of *I. xeranticus* and *P. linteus*.[34] These compounds exhibited significant free radical scavenging activity. Hispidin (**1**) and hypholomine B (**5**) were the most abundant metabolites and were found to be responsible for the anti-oxidative activity of the crude extract. Caffeic acid (**11**), a precursor of hispidin biosynthesis, was found to be highly accumulated in culture broths containing small amounts of hispidin (**1**) and hypholomine B (**5**). The reason for this is still not clear, but may be due to weak activity of the enzymes associated with hispidin biosynthesis. Although biosynthesis of dimeric hispidins from two monomers via oxidative coupling by lignolytic enzymes, laccase and peroxidase, has been proposed, the details of this process remain unknown. In preliminary experiments, we attempted enzymatic synthesis of the hispidin dimer from hispidin (**1**) using commercially available horseradish peroxidase, resulting in production of a hispidin dimer, 3,14′-bihispidinyl (**3**).[35] whereas the other dimers including hypholomine B (**5**) and 1,1-distyrylpyrylethan (**10**) were not detected. This result revealed the dominance of the coupling modes forming the covalent bond at C-3 and C-14′ of two units of hispidin (**1**), and indicated that additional catalysts or substrates are needed for synthesis of other hispidin dimers. This result also suggests that hispidin redox potentials and different types of peroxidase are sufficient for generation of the biosynthetic diversity of styrylpyrones and production of many of its derivatives that have not yet been identified.

Natural anti-inflammatory and anti-arthritic agents against 3α-hydroxysteroid dehydrogenase, cyclooxygenase and xanthine oxidase were isolated from the ethanolic extract of the fruiting body of *Inonotus* sp. and identified as hispidin (**1**), isohispidin (tautomeric γ-pyrone, **12**), 4-(3,4-dihydroxyphenyl)-but-3-en-2-one (**13**), inonotic acid methylester (**14**) and inotilone (**15**).[36] Isohispidin (**12**) co-occurs as a minor compound, together with hispidin (**1**), suggesting that hispidin is a more stable structure than isohispidin (**12**). These metabolites (**1**, **12–15**) share the same biosynthetic origin as polyketides derived from caffeoyl-CoA. The structurally unusual inotilone (**15**) could be the product of decarboxylation-radical ring closure from hispolon (**16**), which was not isolated from this fungus, but is present in *I. hispidus*. Inotilone (**15**) showed immunomodulatory, anti-cancer and anti-viral activity (Figure 2).[37]

A recent study of the chemical constituents of the yellow pigments in the fruiting bodies of *P. igniarius*, *P. linteus*, *I. xeranticus* and *I. obliqqus* afforded unique styrylpyrone derivatives with an unprecedented carbon skeleton. Phelligridins A-J (**17–26**; Figure 2) were isolated from the ethanolic extract of *P. igniarius* together with inoscavin A (**30**), hispolon (**16**), and 4-(3,4-dihydroxyphenyl)-but-3-en-2-one (**13**), and showed anti-oxidative and cytotoxic effects.[38–40] Phelligridins C (**19**) and D (**20**) were then isolated from *P. linteus* and reported as meshimakobnols A and B, respectively.[41] In addition, the fruiting body of *P. linteus* was also reported to produce phellifuropyranone A (**27**) with anti-proliferative activity against mouse melanoma and human lung cancer cells *in vitro*,[42] and two novel furan derivatives, phellinusfurans A (**28**) and B (**29**), which displayed anti-complement activity (Figure 3).[43] Phellifuropyranone A (**27**) was concurrently reported as inoscavin E (**32**), which was isolated from the fruiting bodies of *I. xeranticus*.[44] The novel free radical scavengers, inoscavins A-E (**30–33**, **27**), methylinoscavins A-D (**34–37**) and interfungin A-C (**38–40**) were isolated from the fruiting bodies of *I. xeranticus*, together with phelligridin D (**20**), phelligridin F (**22**), davallialactone (**41**) and methyldavallialactone (**42**) (Figures 3 and 4).[45–49] Compound **41** was first isolated from the rhizome of a fern *Davallia mariesii* and shown to be cytotoxic against BALB/3T3 cells

Table 1 Occurrence of styrylpyrone compounds from medicinal fungi

Compounds	*Producing fungi*	*Biological activity*	*References*
Hispidin (**1**)	*Inonotus hispidus, I. xeranticus, Phellinus igniarius, P. pini, P. ribis, P. linteus, P. baumii*. For details, see ref.10	Anti-oxidant, cytotoxic, anti-inflammatory, anti-viral, anti-dementia	15,20,27–29,34,36,60
Bisnoryangonin (**2**)	*Gymnopilus aeruginosa* For details, see ref.10	Anti-oxidant	20
3,14′-Bihispidinyl (**3**)	*I. hispidus, I. xeranticus, P. linteus, P. ignarius* For details, see ref.10	Anti-oxidant	16,20,30,34,35
Hypholomine A (**4**)	*Hypholoma elongatipes, Pholiota alnicola* For details, see ref.10		20,31
Hypholomine B (**5**)	*Hypholoma elongatipes, Pholiota alnicola, I. hispidus, I. xeranticus, P. linteus, P. ribis* For details, see ref.10	Anti-oxidant, anti-diabetes, anti-inflammatory	16,20,31,34,36,56
Fasciculin A (**6**)	For details, see ref.10		20,31
Fasciculin B (**7**)	For details, see ref.10		20,31
Phelligridimer A (**8**)	*P. igniarius*	Anti-oxidant	32
Squarrosidine (**9**)	*Pholiota squarrosa*	Anti-inflammatory	33,36
1,1-distyrylpyrylethan=Pinillidin (**10**)	*P. linteus, P. pini, I. xeranticus*	Anti-oxidant, anti-inflammatory	33,34,36
Isohispidin (**12**)	*Inonotus* sp.	Anti-inflammatory	36
Phelligridin A (**17**)	*P. igniarius*	Cytotoxic	38
Phelligridin B (**18**)	*P. igniarius*	Cytotoxic	38
Phelligridin C (**19**)=Meshinokobnol A	*P. igniarius, P. linteus*	Cytotoxic	38,41
Phelligridin D (**20**)=Meshinokobnol B	*P. igniarius, P. linteus, P. baumii, I. xeranticus, I. obliquus*	Cytotoxic	38,41,48,51
Phelligridin E (**21**)	*P. igniarius, I. obliquus*	Cytotoxic	38,51
Phelligridin F (**22**)	*P. igniarius, I. xeranticus*	Cytotoxic	38,45
Phelligridin G (**23**)	*P. igniarius, I. obliquus*	Anti-oxidant, cytotoxic	39,51
Phelligridin H (**24**)	*P. igniarius*	Anti-oxidant, cytotoxic	40
Phelligridin I (**25**)=Inonoblin A	*P. igniarius, I. obliquus*	Anti-oxidant, cytotoxic	40,51
Phelligridin J (**26**)	*P. igniarius*	Anti-oxidant, cytotoxic	40
Inoscavin E (**27**)=Phellifuropyranone A	*I. xeranticus, P. linteus*	Anti-oxidant, cytotoxic	42,44
Phellinusfuran A (**28**)	*P. linteus*	Anti-inflammatory	43
Phellinusfuran B (**29**)	*P. linteus*	Anti-inflammatory	43
Inoscavin A (**30**)	*I. xeranticus, P. igniarius, P. linteus*	Anti-oxidant, antidiabetes	45,46,56
Inoscavin B (**31**)	*I. xeranticus*	Anti-oxidant	46
Inoscavin C (**32**)	*I. xeranticus*	Anti-oxidant	47
Inoscavin D (**33**)	*I. xeranticus*	Anti-oxidant	48
Methylinoscavin A (**34**)	*I. xeranticus*	Anti-oxidant	46
Methylinoscavin B (**35**)	*I. xeranticus*	Anti-oxidant	46
Methylinoscavin C (**36**)	*I. xeranticus*	Anti-oxidant	47
Methylinoscavin D (**37**)	*I. xeranticus*	Anti-oxidant	48
Interfungin A (**38**)	*I. xeranticus, P. linteus, P. baumii*	Anti-oxidant, anti-diabetes	49,56,57
Interfungin B (**39**)	*I. xeranticus*	Anti-oxidant	49
Interfungin C (**40**)	*I. xeranticus*	Anti-oxidant	49
Davallialactone (**41**)	*I. xeranticus, P. igniarius, P. baumi*	Anti-oxidant, antidiabetes, anti-inflammatory, anti-platelet aggregation	47,50,56,58,59
Methyldavallialactone (**42**)	*I. xeranticus*	Anti-oxidant, antidiabetes	47,56
Inonoblin B (**43**)	*I. obliquus*	Anti-oxidant	51
Inonoblin C (**44**)	*I. obliquus*	Anti-oxidant	51
Phellinin A1 (**45**)	*Phellinus* sp.	Anti-oxidant	52,53
Phellinin A2 (**46**)	*Phellinus* sp.	Anti-oxidant	52,53

transformed with the H-ras oncogene.[50] Interestingly, compound **41** was a major constituent of the medicinal fungi *I. xeranticus* and *Phellinus* spp., and showed potent and diverse biological activities, which will be discussed in the biological section. New free radical scavengers, inonoblins A-C (**25**, **43**, **44**), were isolated from the methanolic extract of *I. obliquus*, along with phelligridins D (**20**), E (**21**) and G (**23**) (Figure 4).[51] Inonoblin A (**25**) was re-isolated from *P. igniarius* and named phelligridin I. Phellinins A1 (**45**) and A2 (**46**),

unique styrylpyrones, were isolated from the culture broth of *Phellinus* sp. KACC903057P, along with hispidin (**1**) and 1,1-distyrylpyrylethan (**10**).[52,53] Phellinin A was isolated as a mixture of two isomers, A1 (**45**) and A2 (**46**), which could not be separated using a general purification process (Figure 4). Compounds **45** and **46** could be biosynthesized by condensation of *trans*-γ-monocyclofarnesyl pyrophosphate and hispidin (**1**), followed by oxidative and non-stereoselective cyclization. Phellinin A (**45**, **46**) is the first example of condensation of hispidin (**1**) and an isoprene unit, representing a new group of a natural polyketide-isoprenoid hybrid compounds.

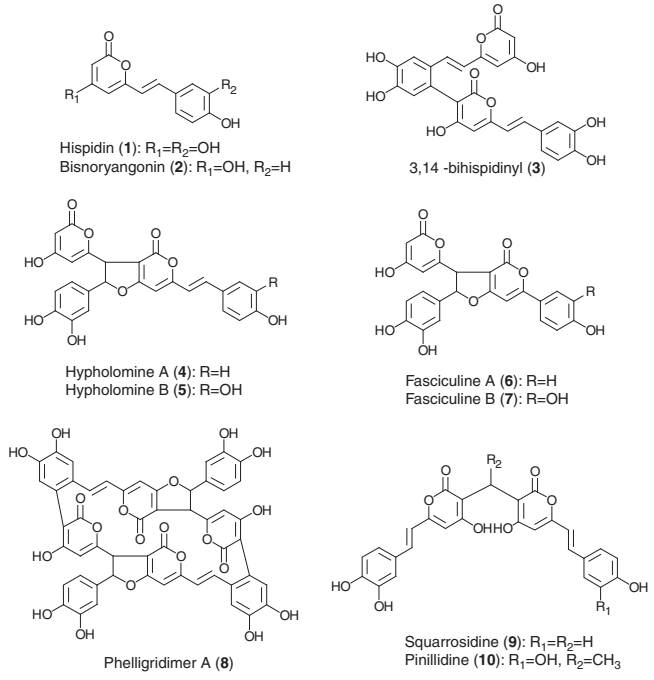

Figure 1 Structures of hispidin (**1**), bisnoryangonin (**2**) and their dimeric and tetrameric compounds.

BIOLOGICAL ACTIVITIES OF STYRYLPYRONES

Anti-oxidant activity

Free radicals have been implicated in pathogenesis of various human diseases, such as myocardial and cerebral ischemia, arteriosclerosis, diabetes, rheumatoid arthritis, inflammation, cancer-initiation and aging processes. Therefore, there is a growing need for free radical scavengers that can be used as protective agents against these diseases.

Styrylpyrones have been reported to have potent anti-oxidant activity. Hispidin (**1**) and its dimers, 3,14′-bihispidinyl (**3**), hypholomine B (**5**) and 1,1-distyrylpyrylethan (**10**), showed significant radical scavenging activity in a concentration-dependent manner,[34] whereas dimeric hispidins possessing two catechol moieties, 3,14′-bihispidinyl (**3**), hypholomine B (**5**) and 1,1-distyrylpyrylethan (**10**), exhibited more potent activity than hispidin (**1**) for DPPH and ABTS radical scavenging activity. Therefore, the anti-oxidative effects of hispidin (**1**), 3,14′-bihispidinyl (**3**), hypholomine B (**5**) and 1,1-distyrylpyrylethan (**10**) against DPPH and ABTS radicals may have originated from the catechol moiety. Their activities are about 2–3 times more potent than those of Trolox, a well-known commercial anti-oxidant.

Inoscavins A-E (**30–33**, **27**), methylinoscavins A-D (**34–37**) and interfungins A-C (**38–40**) from the fruiting body of *I. xeranticus*, and inonoblins A-C (**25**, **44**, **45**) from the fruiting body of *I. obliquus* showed potent free radical scavenging activity (Table 1).[44–49] Among the compounds tested, davallialactone (**41**), which was isolated by our

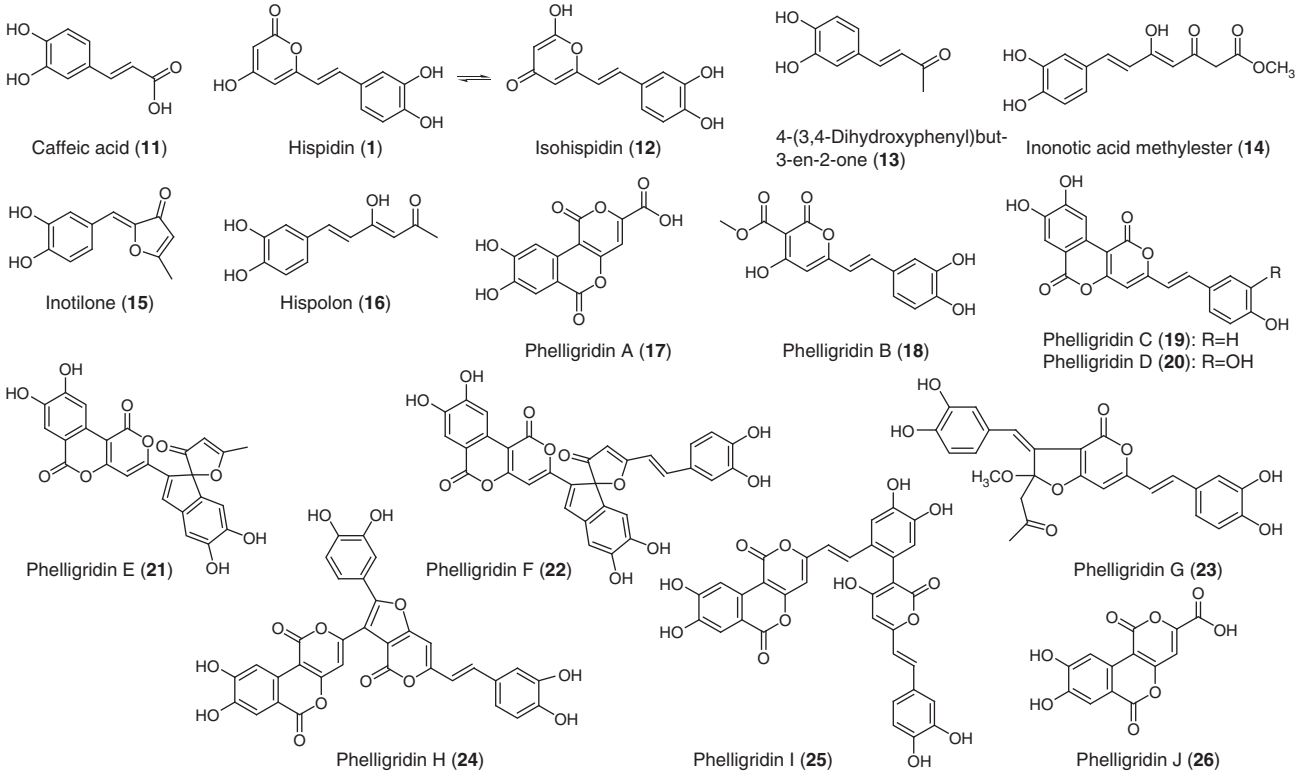

Figure 2 Structures of compounds **1–26**.

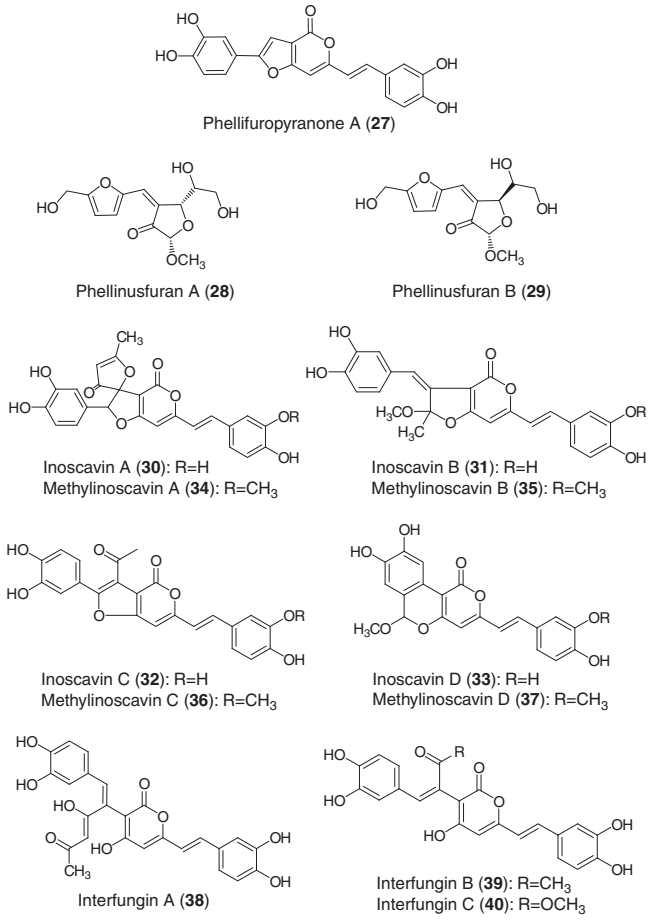

Figure 3 Structures of compounds **27–40**.

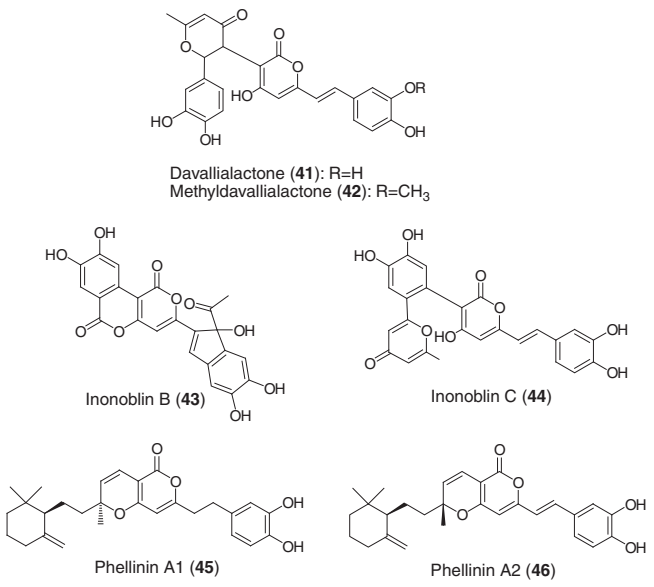

Figure 4 Structures of compounds **41–46**.

group from *I. xeranticus* and *I. obliquus*, was the most potent free radical scavenger. Compounds **41** and **42** displayed significant ABTS radical scavenging activity, with IC_{50} values of 0.8 and 1.5 μM (vitamin E, 5.7 μM), respectively. They showed DPPH radical scavenging activity, with IC_{50} values of 3.4 and 18.7 μM (vitamin E, 12.3 μM), respectively, and superoxide radical scavenging activity, with IC_{50} values of 2.3 and 5.4 μM (vitamin E, >100 μM; caffeic acid, 2.9 μM). Compound **41** is about 2–5 times more potent than compound **42,** in which the hispidin moiety was masked by a methyl group. Inoscavin A inhibited rat liver microsomal lipid peroxidation, with an IC_{50} value of 0.3 μg ml^{-1}, which is five times the potency of vitamin E (1.5 μg ml^{-1}). Phelligridimer A (**8**) and Phelligridins G-J (**23–26**) from the fruiting body of *P. igniarius* inhibited rat liver microsomal lipid peroxidation, with IC_{50} values of 10.2, 3.8, 4.8, 3.7 and 6.5 μM, respectively.[32,39,40]

Cytotoxicity

Hispidin (**1**) inhibited isoform-β of protein kinase C (IC_{50} 2 μM), which is more cytotoxic toward cancerous cells (pancreatic duct and keratinocyte) than normal cells (fibroblast).[27] Hispolon (**16**) from *P. linteus* showed dose-dependent inhibition of human epidermoid KB cell proliferation, with an IC_{50} value of 4.62 μg ml^{-1}, and induced cell death in KB cells through a mitochondria-mediated apoptotic pathway.[54] Further research on the mechanism by which compound **16** induces apoptosis was recently conducted. Downregulation of the MDM2 proto-oncogene, which is overexpressed in many human tumors, has been an attractive therapeutic strategy for treatment of cancer. Compound **16** displayed anti-proliferative effects via MDM2-recruited ERK1/2 activity in breast and bladder cancer cells.[55] Phelligridin A-J (**17–26**) from *P. igniarius*, and phellifuropyranone A (**27**) from *P. linteus* showed modest *in vitro* cytotoxicity against several human cancer cell lines.[38–40,42]

Anti-diabetic activity

Diabetes mellitus is a leading cause of many complications, such as artherosclerosis, cardiac dysfunction, retinopathy, neuropathy and nephropathy. Hyperglycemia may have an important role in pathogenesis of diabetic complications through several mechanisms, such as increased aldose reductase-related polyol pathway flux and increased formation of advanced glycation end products. Thus, inhibition of aldose reductase and protein glycation is effective for treatment of diabetic complications. The fruiting body of *P. linteus* showed inhibitory activity against both.[56,57] Davallialactone (**41**), methyldavallialactone (**42**), hypholomine B (**5**), interfungin A (**38**) and inoscavin A (**30**) exhibited potent inhibitory activity against rat lens and human recombinant aldose reductases, with IC_{50} values of 0.33, 0.51, 0.82, 1.03, 1.06 μM and 0.56, 1.15, 1.28, 1.82, 1.40 μM, respectively. Interfungin A (**38**) was tested as a potential compound for inhibition of protein glycation and has demonstrated potent inhibition of cross-linking of proteins, which was more effective than aminoguanidine, a well-known inhibitor of advanced glycation end products.

Anti-inflammatory activity

Inflammation is a multifaceted response mediated by activation of cells in the immune system. Of these cells, macrophages have a central role in many pathological processes during inflammation, including overproduction of inflammatory mediators, such as NO by inducible NOS (iNOS) and prostaglandin E_2 by cyclooxygenase-2, and increased expression of cell surface molecules, such as CD80 and CD86. In activated RAW264.7 cells, davallialactone (**41**) strongly downregulated the LPS-mediated inflammatory response, including NO production, prostaglandin E_2 release, expression of the proinflammatory cytokine gene and cell surface expression of co-stimulatory molecules. Treatment with compound **41** did not alter cell viability or morphology. Compound **41** was found to exert its anti-inflammatory effects through inhibition of a signaling cascade that activates nuclear factor

κB via P13 K, Akt and IKK, but not mitogen-activated protein kinases.[58] Treatment with **41** affected phosphorylation of these signaling proteins, but not their level of expression. These inhibitory effects were not due to interruption of toll-like receptor 4 binding to CD14. Compound **41** also inhibited LPS-induced phosphorylation and kinase activity of Src, implying that Src may be a potential pharmacological target of davallialactone (**41**).[58]

Hispidin (**1**) and inotilone (**15**) selectively inhibited cyclooxygenase-2 at concentrations as low as those of the standard inhibitors, meloxicam and nimesulide. Hispidin (**1**) also showed good 3α-hydroxysteroid dehydrogenase and xanthine oxidase inhibitory activities.[36] For the tautomeric hispidin and isohispidin (**12**), α-pyrone appeared to be more active than γ-pyrone. Compared with allopurinol (IC_{50} 4.4 μM), hypholomine B (**5**), squarrosidine (**9**) and 1,1-distyrylpyrylethan (**10**) proved to be potent inhibitors of xanthine oxidase, with IC_{50} values of 6.7, 8.1 and 5.8 μM, respectively.

The complement system is a major effector of humoral immunity, and modulation of complement activity can be important for treatment of inflammation. Phellinusfurans A (**28**) and B (**29**) exhibited significant inhibitory activity in the classical pathway (CP) of the complement system, with IC_{50} values of 33.6 and 33.7 μM, respectively, comparable to the positive control, rosmarinic acid (IC_{50} 180 μM).[43]

Anti-platelet aggregation activity

Davallialactone (**41**) dose-dependently inhibited platelet aggregation stimulated either by collagen or by thrombin, and induced by ADP. In addition, it inhibited intracellular calcium concentration level, phosphorylation of extracellular signal-regulated protein kinase (ERK)-2 and p38 mitogen-activated protein kinase (MAPK) in a dose-dependent manner. Tyrosine phosphorylation of 60 and 85 kDa proteins activated by collagen were shown to be differentially inhibited by **41**. Thus, compound **41** may have potential anti-platelet aggregation activity via suppression of intracellular downstream signaling pathways.[59]

Anti-viral activity

Hispidin (**1**) and hispolon (**16**) showed considerable anti-viral activity against influenza viruses type A (H1N1 and H3N2) and B using the allantois on the shell-test system.[15] HIV-1 integrase is one of the three enzymes critical to viral replication. The other two are reverse transcriptase and protease. Development and therapeutic administration of inhibitors of the latter two enzymes had a significant effect on control of the spread of HIV infection. However, emergence of multi-drug-resistant viruses, even in drug-naïve patients, has appeared; thus, inhibition of HIV-1 integrase is one of the most promising new targets for anti-retroviral therapy. Hispidin (**1**) showed HIV-1 integrase inhibitory activity, with an IC_{50} value of 2 μM in the coupled assay system. Inhibition of HIV-1 integrase was completely abolished when the phenolic groups of hispidin were capped with a methyl ether, indicating that the acidic phenolic groups are critical to integrase inhibitory activity, a known phenomenon of this enzyme.[60]

Anti-dementia activity

Alzheimer's disease is a neurodegenerative disorder; a major histopatholgical characteristic of this disease is the deposition of amyloid proteins (amyloid plaques) in the parenchyma of the amygdale, hippocampus and neocortex. β-amyloid peptide (Aβ), which is formed by α-, β-, and γ-secretase through cleavage of the amyloid precursor protein, is the major component of amyloid plaques. Among these secretases, BACE1 (β-site amyloid precursor protein cleaving enzyme) is at present the most attractive target for inhibition of amyloid production. Thus, BACE1 inhibitors should reduce Aβ levels. Hispidin (**1**) from the mycelial culture of *P. linteus* noncompetitively inhibited BACE1 in a dose-dependent manner, showing an IC_{50} value of 4.9 μM. In addition, hispidin (**1**) appeared to be a relatively specific inhibitor of BACE1 and prolyl endopeptidase, as it showed no activity against TACE (tumor necrosis factor alpha converting enzyme) and other serine proteases, such as chymotrypsin, trypsin and elastase.[28]

BIOSYNTHESIS OF STYRYLPYRONES

Biosynthesis of hispidin (**1**) has been studied at labeling experiment level; however, that for other styrylpyrones remains postulative. Incorporation of [1-^{14}C]acetate and [U-^{14}C]L-Phe into **1** in *Polyporus schweinizii* indicated that it is derived from phenylpropanoid and two acetate units. Tracer studies showed incorporation of D,L-Phe (**47**), D,L-tyrosine (**49**), cinnamate (**48**), *p*-coumarate (**50**) and caffeate (**11**) into the styryl unit, and efficient incorporation of acetate and malonate into the pyrone ring.[25] In addition, *p*-coumaric acid hydroxylase in *I. hispidus* has been reported to exist as two forms, E1 and E2.[61] These enzymes were capable of hydroxylation of *p*-coumaric acid (**50**) and bis-noryangonin (**2**) into caffeic acid (**11**) and hispidin (**1**), respectively, suggesting a potential alternative route for synthesis of hispidin from *p*-coumaric acid (**50**) without involvement of caffeic acid (**11**) as an intermediate (Figure 5). Thus, it has been suggested that bis-noryangonin (**2**) may not be found naturally in *Phellinus* and *Inonotus* spp., and that this metabolite exists mainly in *Gymnopilus* and *Pholiota* spp. rather than *Phellinus* and *Inonotus* spp. Another biosynthetic pathway mediated by 4-hydroxy-6-methyl-2-pyrone (**51**), which is formed by the reaction of three units of acetyl-CoA and one 3,4-dihydroxybenzoyl-CoA (3,4-dihydroxy benzaldehyde) (**52**), has been proposed; this pathway resulted from co-occurrence of **52** and 3,4-dihydroxybenzoic acid with styrylpyrone metabolites (Figure 6).[38]

Light is required for hispidin biosynthesis in *I. hispidus* in order to initiate a sequential increase in the activity of enzymes involved in production of styrylpyrones.[62,63] Not only pigment formation, but also cinnamate and *p*-coumarate hydroxylase activity and tyrosine ammonia-lyase and aminotransferase activity for Phe were stimulated by light. These findings were supported by results from a previous study, in which production of phenolic compounds by *I. obliquus* was enhanced under oxidative stress. Yield and composition of polyphenols, including phelligridins and inoscavins, can be considerably increased or extensively modified by addition of H_2O_2 or H_2O_2 and arbutin.[64] These conditions affected the diversity of styrylpyrones found in wild mushroom and mycelial cultures in different ways. Metabolites isolated from wild mushrooms have been reported to have greater complexity with greater structural diversity than those from the mycelial cultures or cultivated mushrooms, which are more vulnerable to microbial attacks, ultraviolet radiation and other conditions. It has been proposed that the polyphenols from the wild mushrooms are biosynthesized mainly via oxidative coupling of the precursors hispidin (**1**) and hispolon (**16**), or 3,4-dihydroxyphenyl-propanoids by mushroom peroxidase (Figures 7 and 8). On the other hand, hispidin (**1**) and its dimer are the metabolites isolated from the mycelial cultures or cultivated mushrooms; the dimer is generated by the oxidative coupling of two units of hispidin (**1**), specifically through condensation of dehydrohispidin by the lignino-lytic enzyme (Figure 10).[34]

As shown in Figures 7 and 8, hispolon (**16**) and 4-(3,4-dihydroxyphenyl)-3-buten-2-one (**13**) might be biosynthesized by coupling

of either 3,4-dihydroxybenzaldehyde and two or three acetates, or cinnamoyl-CoA and one or two acetates, followed by decarboxylation. Interfungins A (**38**) and B (**39**) might be biogenerated by condensation of **1** and **16** and **1** and **13**, respectively; this process may be catalyzed by mushroom peroxidase. The natural and/or enzymatic rearrangement of the highly functionalized metabolites **38** and **39** may provide mechanistic insight for formation of mushroom polyphenols, such as inoscavins A-C (**30–32**), davallialactone (**41**) and phelligridin F (**22**) (Figures 7 and 8).[49]

Compounds containing the 8,9-dihydroxy-1*H*,6*H*-pyrano[4,3-*c*][2]benzopyran-1,6-dione moiety could be formed by coupling of three units of acetyl-CoA and 3,4-dihydroxy benzoic acid or 3,4-dihydroxybenzaldehyde. Further coupling of hispidin with 4-hydroxybenzoic acid or 3,4-dihydroxybenzoic acid would generate phelligridin C (**19**) or phelligridin D (**20**), and coupling of hispidin with 3,4-dihydroxybenzaldehyde (**52**) would give inoscavin D (**33**) (Figure 9).[48] These metabolites could also be produced by coupling of hispidin or bis-noryangonin with 3,4-dihydroxybenzoyl-CoA. Phelligridin E (**21**) might be formed via coupling of phelligridin D (**20**) with 4-hydroxy-6-methyl-2-pyrone (**51**), and further reaction by phelligridin E (**21**) and 3,4-dihydroxybenzaldehyde (**52**) would generate phelligridin F (**22**).[39]

Hispidin (**1**) may be transformed into more elaborate metabolites by coupling with a second pyrone. Mycelial cultures of *Phellinus* and *Inonotus* sp. produced hispidin (**1**) and its dimers, 3,14′-bihispidinyl (**3**), hypholomine B (**5**) and 1,1-distyrylpyrylethan (**10**), which could be regarded as condensation products of dehydrohispidin. In addition, the mechanism of dipyrone formation for phelligridimer A (**8**), squarrosidine (**9**), and fasciculins A (**6**) and B (**7**) could be similar. Formation of 3,14′-bihispidinyl (**3**) was proposed by initial oxidation of hispidin (**1**) to dehydrohispidin, followed by conjugative attack at the ortho-quinone by the nucleophilic (C-3) center of the pyrone ring in a second hispidin (Figure 10).[20] Hypholomines A (**4**) and B (**5**) might result from attack at the electrophilic double bond by the nucleophilic C-3 of the pyrone ring in hispidin (**1**) and bis-noryangonin (**2**), respectively, followed by formation of the dihydrofuran ring.[10] However, hypholomine A was not detected due to deficiency of bis-noryangonin (**2**) in *Phellinus* and *Inonotus* spp. Phelligridimer A (**8**) may be sequentially or simultaneously formed from oxidative coupling of four hispidins and/or from two units of hypholomine B (**5**).[32] Bis-styrylyrones squarrosidine (**9**) and 1,1-distyrylpyrylethan (**10**) represent new members of the fungal phenylpropanoids with an unprecedented 3,3′-fusion. Their formation can be rationalized by nucleophilic vinylogous addition of the enol of hispidin (**1**) to a tautomeric form of a methylated hispidin derivative, followed by methylation of the methylene bridge, generating 1,1-distyrylpyrylethan (**10**).[33] Fasciculins A (**6**) and B (**7**) from *Hypholoma* and *Pholiota* sp. presumably arise by condensation with 6-(4′-hydroxyphenyl)-4-hydroxy-2-pyrone and 6-(3′,4′-dihydroxyphenyl)-4-hydroxy-2-pyrone, respectively.[20] Nevertheless, the arylpyrones implicated in biosynthesis of fasciculins have not yet been isolated from *Phellinus* and *Inonotus* sp.

Yangonin (**55**), a 4-methoxy-6-(4-methoxystyryl)-2-pyrone, was first isolated from the plant *Piper methysticum*, which has been used in treatment of anxiety, nervous tension, agitation and insomnia.[18,65] Its pharmacological effects are associated with a group of styrylpyrones termed kavapyrones, including kawain (**53**), dihydrokawain (**54**),

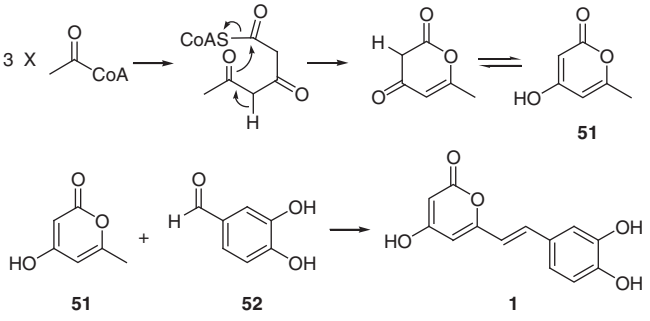

Figure 6 Proposed biosynthetic pathways of hispidin (**1**).

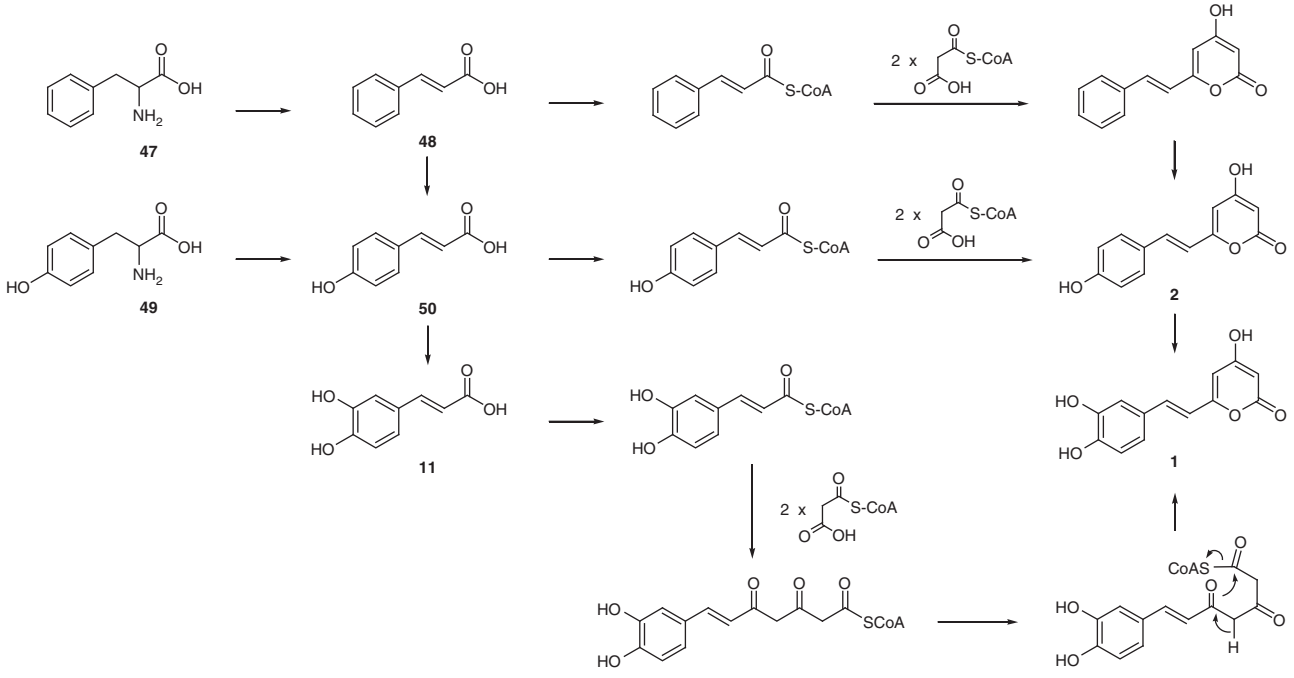

Figure 5 Proposed biosynthetic pathways of hispidin (**1**).

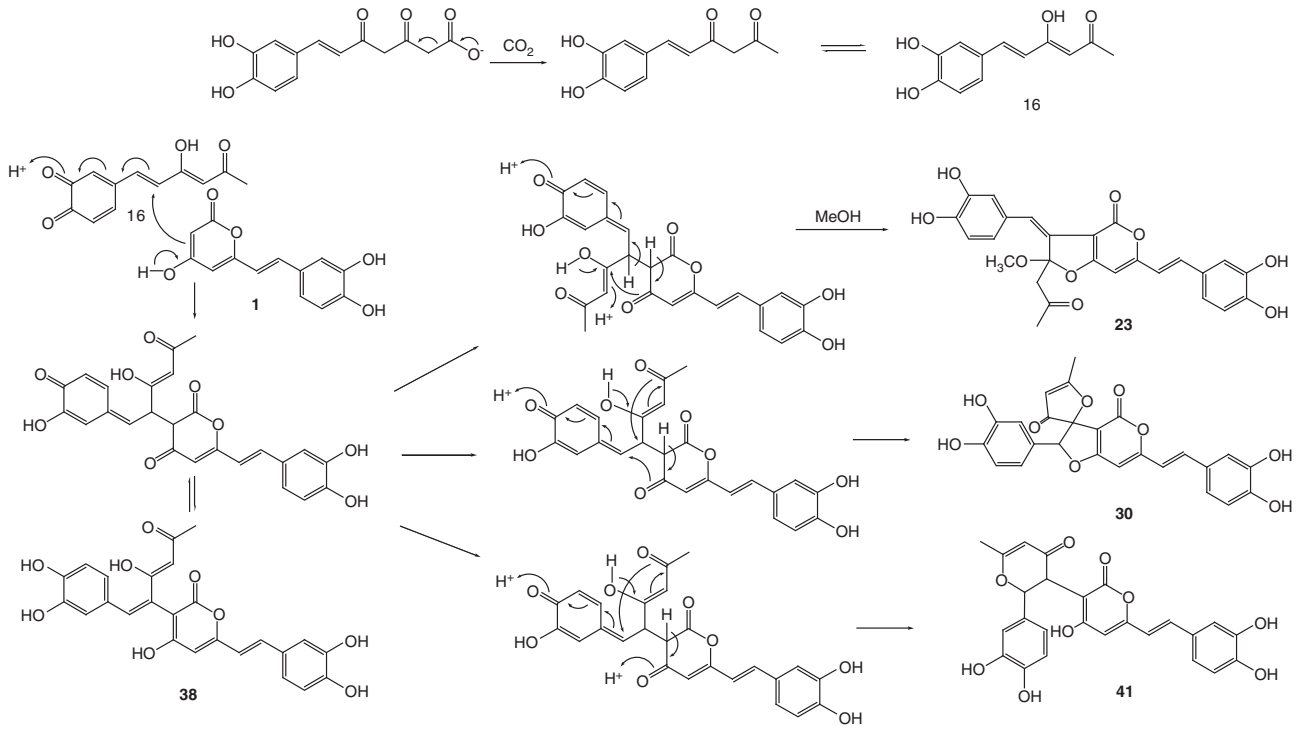

Figure 7 Proposed biosynthetic pathways of phelligridin G (**23**), inoscavin A (**30**) and davallialactone (**41**).

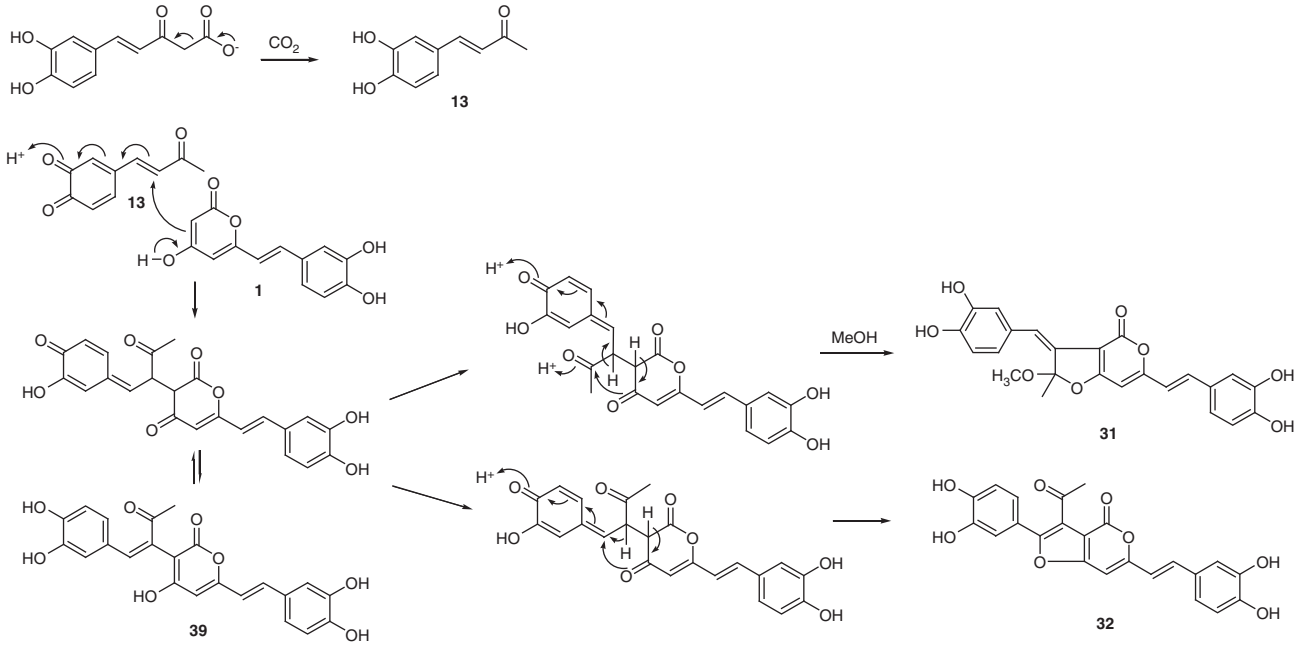

Figure 8 Proposed biosynthetic pathways of inoscavins B (**31**) and C (**32**).

yangonin (**55**), demethoxyyangonin (**56**), methysticin (**57**) and dihydromethysticin (**58**) (Figure 11). The C-4 methyl group on the pyrone ring, which is different from that of fungal styrylpyrones, is the common characteristic of these kavapyrones. Biosynthesis of styrylpyrone has been extensively studied in the plant *Equisetum arvense*, in which styrylpyrones and flavonoids constitutively accumulate in distinct organs, and styrylpyrones and caffeic acid derivatives may act as effective resistance factors, which appear to have similar ecological and physiological roles as fungal styrylpyrones.[17] Equisetumpyrone (**59**), a styrylpyrone glucoside in gametophytes from *Equisetum arvense*, differs in the oxygenation pattern of its pyrone ring from the fungal styrylpyrones, which are never oxygenated at

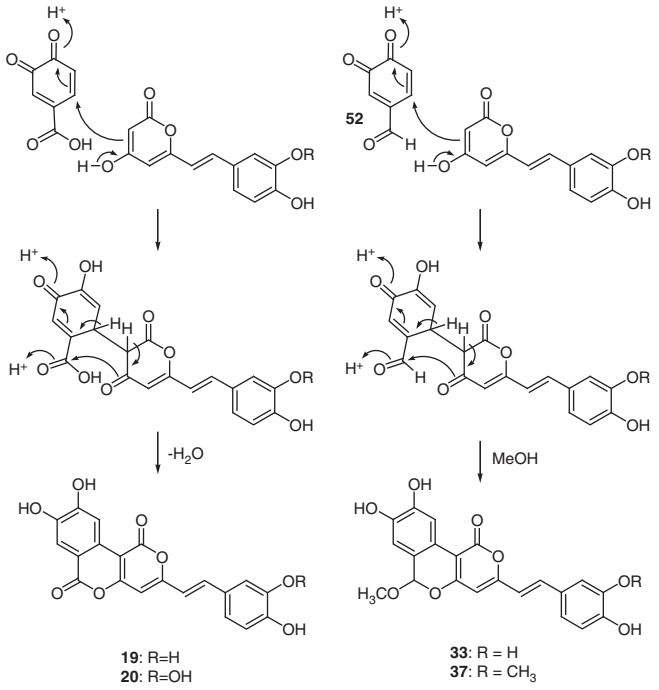

Figure 9 Proposed biosynthetic pathways of phelligridins C (**19**) and D (**20**), inoscavin D (**33**) and methylinoscavin D (**37**).

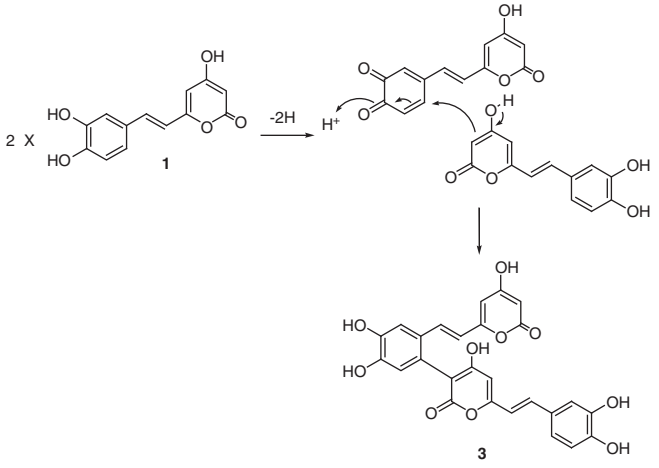

Figure 10 Proposed dimerization mechanism of hispidin (**1**) into 3,14′-bihispidinyl (**3**) by horseradish peroxidase.

C-3.[66,67] Moreover, hispidin 4-*O*-β-D-glucopyranoside (**60**), an inhibitor of both low-density lipoprotein oxidation and ROS production, is glycosylated at C-4 (Figure 11).[68] In *Phellinus* and *Inonotus* spp., however, no glycosides of styrylpyrones have been found. Thus, it appears that styrylpyrones originating from plants and fungi are biosynthesized from a similar pathway, but possess different structural features, possibly due to environmental differences.

CONCLUSIONS

The fruiting bodies of *P. linteus*, *P. igniarius*, *P. ribis*, *I. obliquus* and *I. xeranticus*, belonging to the genera *Phellinus* and *Inonotus*, have been used as traditional medicines in Asian countries. Polysaccharides,

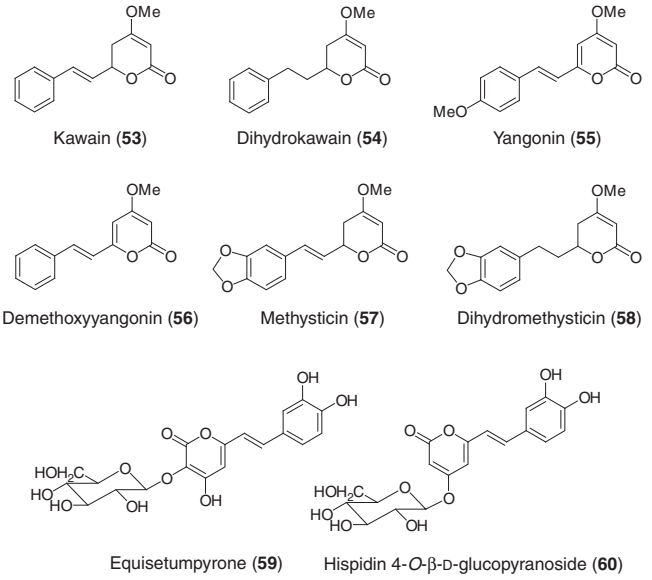

Figure 11 Structures of compounds **53–60**.

particularly β-glucan, have received significant attention, and a number of polysaccharides and protein-bound polysaccharides from medicinal mushrooms have been developed for treatment of cancer. Krestin, Mesima, Lentinan and Schizophylan are clinically used representatives. However, there is little evidence to show that polysaccharides are solely responsible for the biological effects of medicinal mushrooms. The biological effects of the methanolic extract of the fruiting bodies of these medicinal fungi have been reported; however, only a few studies have reported on the active principles of the extract. Many research groups have begun identification of the active ingredients of medicinal mushrooms. In addition, researchers have also attempted to determine the chemical constituents, aside from polysaccharides, that are responsible for the biological activity of medicinal mushrooms, with a particular focus on the yellow polyphenol pigments (Table 1). Styrylpyrone-type polyphenol pigments showed various biological activities, including anti-oxidative, anti-inflammatory, cytotoxic, anti-platelet aggregation, anti-diabetic, anti-dementia and anti-viral effects. Unfortunately, these mushrooms are rare in nature; consequently, their use is restricted. Therefore, cultivation and mycelial fermentation have been developed for mushrooms.[69–71] Styrylpyrone composition of wild-grown and cultivated *P. linteus* and the cultured mycelia differed from each other.[72] This may be attributed to environmental factors, such as microbial attack, ultraviolet radiation and oxidative stress. Nevertheless, the cultivated mushroom or cultured mycelia of *P. linteus* exhibited biological activities comparable to the wild-grown mushroom, and produced an abundant amount of yellow pigments containing mainly styrylpyrones with various biological activities.[28,29,34,52–57,60,64,72–75] In addition, the adverse effects of the medicinal mushrooms *Phellinus* and *Inonotus* have not been reported until now. Methanolic extracts of *I. xeranticus* did not show acute toxicity up to $2\,g\,kg^{-1}$ in mice, and the LD_{50} values of these extracts were above $2\,g\,kg^{-1}$, indicating the safe nature of these mushrooms.[76] Therefore, medicinal mushrooms *Phellinus* and *Inonotus* hold great promise for use in pharmacological applications. This is especially true for the styrylpyrone class of yellow pigments, which might be a good candidate for use in drug discovery.

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