Compounds from Wild Mushrooms with Antitumor Potential

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Abstract: For thousands of years medicine and natural products have been closely linked through the use of traditional medicines and natural poisons. Mushrooms have an established history of use in traditional oriental medicine, where most medicinal mushroom preparations are regarded as a tonic, that is, they have beneficial health effects without known negative side-effects and can be moderately used on a regular basis without harm. Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of compounds which are modulators of tumour cell growth. Furthermore, they may have potential as functional foods and sources of novel molecules. We will review the compounds with antitumor potential identified so far in mushrooms, including low-molecular-weight (LMW, e.g. quinones, cerebrosides, isoflavones, catechols, amines, triacylglycerols, sesquiterpenes, steroids, organic germanium and selenium) and high-molecular-weight compounds (HMW, e.g. homo and heteroglucans, glycans, glycoproteins, glycopeptides, proteoglycans, proteins and RNA-protein complexes).

Keywords: Mushrooms, antitumor, low-molecular-weight compounds, high-molecular-weight compounds.

1. INTRODUCTION

Over recent decades consumer demands in the field of food production have changed considerably. Consumers increasingly believe that foods contribute directly to their health and well-being. Today's foods are not only intended to satisfy hunger and provide the required nutrients but also to prevent diseases and improve physical and mental well-being of consumers. In this context, functional foods play a very significant role. The increasing demand for such foods can be explained by the rapid advances in science and technology, increasing healthcare costs, changes in food laws affecting label and product claims, the steady increase in life expectancy, the desire of older people for improved quality of life in their later years and rising interest in maintaining good health through diet [1].

According to the Institute of Medicine's Food and Nutrition Board, "Functional Foods" are foods or dietary components that may provide a health benefit beyond basic nutrition. We can take greater control of our health through the food choices we make, knowing that some food can provide specific health benefits [2].

Functional foods could not exist without nutraceutical compounds, the bioactive compounds that give functional properties to food. A nutraceutical can be defined as a substance that may be considered a food or part of a food and may possibly provide medical or health benefits such as the prevention and treatment of disease. Nutraceuticals may range from isolated nutrients and dietary supplements to genetically engineered "designer" foods, herbal products and processed products such as cereals, soups and beverages [3].

When considering natural species as sources of compounds with potential medicinal properties, Baker *et al*. [4] suggested that one should take into account the: i) evidence regarding the traditional usage of the substance by indigenous populations, ii) abundance of the species in nature and iii) sustainable utilisation of the species. These criteria were suggested for the discovery of natural products from the Plant Kingdom. However, fungi, a separate Kingdom from plants, could also complete these criteria and most importantly, the sustainability of fungi can be achieved by using artificial cultivation techniques. Another extremely important criterion when searching for novel bioactive compounds is the uniqueness of the organism and its potential to produce secondary metabolites [5]. In fact, in addition to the ancient utilization of plants, the medicinal use of mushrooms also has a very long tradition. Among the large resources of fungi, higher Basidiomycetes, especially mushrooms, represent unlimited sources of therapeutically useful biologically active agents [6-8]. In particular, mushrooms with potential useful properties against cancers of the stomach, esophagus, lungs, etc. are known in China, Japan, Korea, Russia, United States and Canada [9].

The number of mushroom species on earth is estimated to be 140,000, suggesting that only 10% are already known. Assuming that the proportion of useful mushrooms among the undiscovered and unexamined mushrooms will be only 5% (a small logical %), this implies 7,000 yet undiscovered species of possible benefit to mankind [10]. The higher Basidiomycetes include about 10,000 species from 550 genera and 80 families in the Basidiomycetes class with macroscopic fruiting bodies. Furthermore, approximately 700 species of higher Basidiomycetes have been found to possess significant pharmacological activities [6,7,9]. The macrofungi have been divided into four groups: edible flesh, medicinal, poisonous and miscellaneous, where the properties are less well defined [11].

It is not surprising that mushrooms are a source of many biologically active compounds. Mushrooms manage to grow in darkness and dampness in highly competitive environments and protect themselves from hordes of attacking microbes by developing natural protective substances. Modern scientific studies on the above called "medicinal mushrooms" have expanded exponentially during the last two decades and scientific explanation to show how compounds derived from mushrooms function in humans are increasingly being established [12]. Fungal fruiting bodies, fungal mycelium or the culture fluid in which the mycelium has been cultivated may all be explored for biological activity. More recently, some species of edible higher Basidiomycetes have been found to markedly inhibit the growth of different tumor cell lines. There are approximately two hundred species of higher Basidiomycetes that were found to have this activity [13]. Additionally, both cellular components and secondary metabolites of a large number of mushrooms have been shown to affect the immune system and therefore might be used to treat a variety of diseases [9]. Mushrooms which appear to enhance or potentiate resistance to disease states are being

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sought for the treatment of cancer, immunodeficiency diseases (including AIDS) or generalized immunosuppression after drug treatment [14,15].

The above named "medicinal mushrooms" have an established history of use in traditional oriental medicine, where most medicinal mushroom preparations are regarded as a tonic, that is, they are claimed to have beneficial health effects without known negative side-effects and can apparently be moderately used on a regular basis without harm. Often, blends of various medicinal mushrooms are used for maximum benefit. Many traditionally used mushrooms from genera, *Auricularia*, *Flammulina*, *Ganoderma*, *Grifola*, *Hericium*, *Lentinus* (Lentinula), *Pleurotus*, *Trametes* (Coriolus), *Schizophyllum*, and *Tremella* have been demonstrated to possess significant medicinal potential [7,16]. *Pleurotus eryngii*, *Lyophyllum shimeji*, *Flammulina velutipes* and *Grifola frondosa* are edible mushrooms that have all been found to contain medicinally active compounds with potential benefit to health. However, many edible species of potent medicinal mushrooms such as *Ganoderma lucidum*, *Trametes versicolor* and *Inonotus obliquus* are very bitter and/or hard to eat and are used in the form of an extract, tea or powder [8,17]. From the edible mushroom species, *Agaricus* are the leader in world production, whilst from the non-edible medicinal species, *Ganoderma* (which belongs to the polypores) are the leader in terms of world production. Several mushroom species belonging to the *Polyporaceae* family are now being regarded as the next candidate producers of possible valuable medicines [6].

The spectrum of detected pharmacological activities of-Basidiomycetes is very broad; among their biological effects, the following have been suggested: antifungal, anti-inflammatory, antitumor, antiviral, antibacterial, antiparasitic, immunomodulating and hepatoprotective; equally promising is their suggested role in the regulation of blood pressure, as well as in the cure of cardiovascular disorders, in hypercholesterolemia and diabetes [18]. Even among the known species, the proportion of well-investigated mushrooms is very low. This fact, together with the knowledge about the great potential of microscopic fungi for production of bioactive metabolites [e.g. *Penicillium*, *Aspergillus*, *Tolypocladium inflatum* W. Gams, *Claviceps purpurea* (Fr.) Tul.], the experience in ethnomedicinal use of mushrooms, the ecologic need for fungi to produce bioactive secondary metabolites and the improved possibilities for genetic, pharmacological and chemical analysis led to the assumption that mushrooms have great potential for successful bioprospecting [19].

In summary, wild mushrooms have been widely used as human food for centuries and have been appreciated for their texture and flavors as well as some suggested medicinal and tonic attributes. However, the awareness of mushrooms as a healthy food and as an important source of biologically active substances with medicinal potential has only recently emerged. Various activities of mushrooms have been studied which include antibacterial, antifungal, antioxidant, antiviral, antitumor, cytostatic, immunosuppressive, antiallergic, antiatherogenic hypoglycemic, anti-inflammatory and hepatoprotective activities [19]. In this review we have collected evidence of mushrooms and compounds extracted from mushrooms which have potential antitumor properties.

2. MUSHROOMS AS MODULATORS OF THE CARCINOGENIC PROCESS

Carcinogenesis is a process which normally takes several years during which progressive genetic changes occur leading to malignant transformation. Cancer prevention is the best intervention in this process before invasive disease develops. Over the last half century, our understanding of carcinogenesis has grown enormously, owing largely to recent technology, allowing exploration of molecular pathways, cancer-associated genes and tissue architecture. This knowledge provided the basis for most cancer-preventive intervention strategies and particularly for one of the strategies, chemoprevention – the use of drugs, biologicals and nutrients to prevent the development of cancer (i.e. to inhibit, delay or reverse carcinogenesis) [20]. Carcinogenesis has traditionally been understood as having three stages: initiation, promotion, and progression. Although more recently carcinogenesis has not been described in those terms, but rather as a malignant transformation as a whole, we hereby refer to the literature in which such divisions of the stages of carcinogenesis were made. It has been described that the modulation of the human immune system, attributed to mushrooms, particularly to various mushroom polysaccharides, was likely to affect primarily the promotion and progression stages, according to the referred model of carcinogenesis. Nonetheless, other substances contained in mushrooms were described as possibly being able to interfere with the referred tumor initiation process, through a variety of mechanisms such as enhancing the antioxidant capacity of cells or upregulating phase I and II enzymes involved in the metabolic transformation and detoxification of mutagenic compounds. Additionally, other mushroom constituents have been described as being able to inhibit what was considered to be the promotion or progression stages of carcinogenesis, by exerting direct cytotoxicity against tumor cells, interfering with tumor angiogenesis, or upregulating other nonimmune tumor-suppressive mechanisms [21].

Many species of Fungi from the division Basidiomycetes (mushrooms) have been found to contain medicinally active compounds, which has been of great recent interest [8,12,21-26]. There are advantages of using mushrooms as sources of bioactive compounds, rather than plants. For example, the fruiting body can be produced in much less time and the mycelium may also be rapidly produced (in a liquid culture that can be manipulated to produce optimal quantities of active products, or from mycelial biomass and supernatant of submerged cultures using bioreactors) [27-31].

3. COMPOUNDS FROM MUSHROOMS WITH ANTITUMOR POTENTIAL

Mushrooms comprise a vast and yet largely untapped source of powerful new pharmaceutical products. In particular, and most importantly for modern medicine, they represent an unlimited source of compounds with potential antitumor and immunostimulating properties, including low-molecular-weight (LMW, e.g. quinones, cerebrosides, isoflavones, catechols, amines, triacylglycerols, sesquiterpenes, steroids, organic germanium and selenium) and high-molecular-weight compounds (HMW, e.g. homo and heteropolysaccharides, glycoproteins, glycopeptides, proteins, RNA-protein complexes) Fig**.** (**1**). We hereby review each of these compounds and their described potential antitumor properties.

3.1. Low Molecular Weight Compounds with Antitumor Potential

Mushrooms contain a variety of complex compounds derived from secondary metabolism such as phenolic compounds, polyketides, triterpenoids and steroids which are specific to each mushroom and have specific effects in humans [8,12,21,25,32]. Many such compounds have been used in the treatment of many health problems, including cancer [33].

The secondary metabolites with LMW present in mushrooms and which have revealed antitumor properties include quinones, cerebrosides, isoflavones, catechols, amines, triacylglycerols, sesquiterpenes, steroids, organic germanium and selenium Table **1**; Fig. (**2**).

Two epoxy compounds derivatives of quinones (**1** and **2**) are involved in modulating the activity of NF-kB, which is implicated in tumor growth [12]. Clavilactones (**3a,b**- quinones and **3c**-hydroquinone) are fungal metabolites endowed with an unsual structure, based on a 10-membered macrolide fused to a benzoquinoid ring and a 2,3-epoxy-γ-lactone [34,35]. They were isolated from the culture medium of the non-toxigenic basidiomycetae *Clitocybe clavipes*. These compounds were identified as inhibitors of protein tyrosine kinases [12].

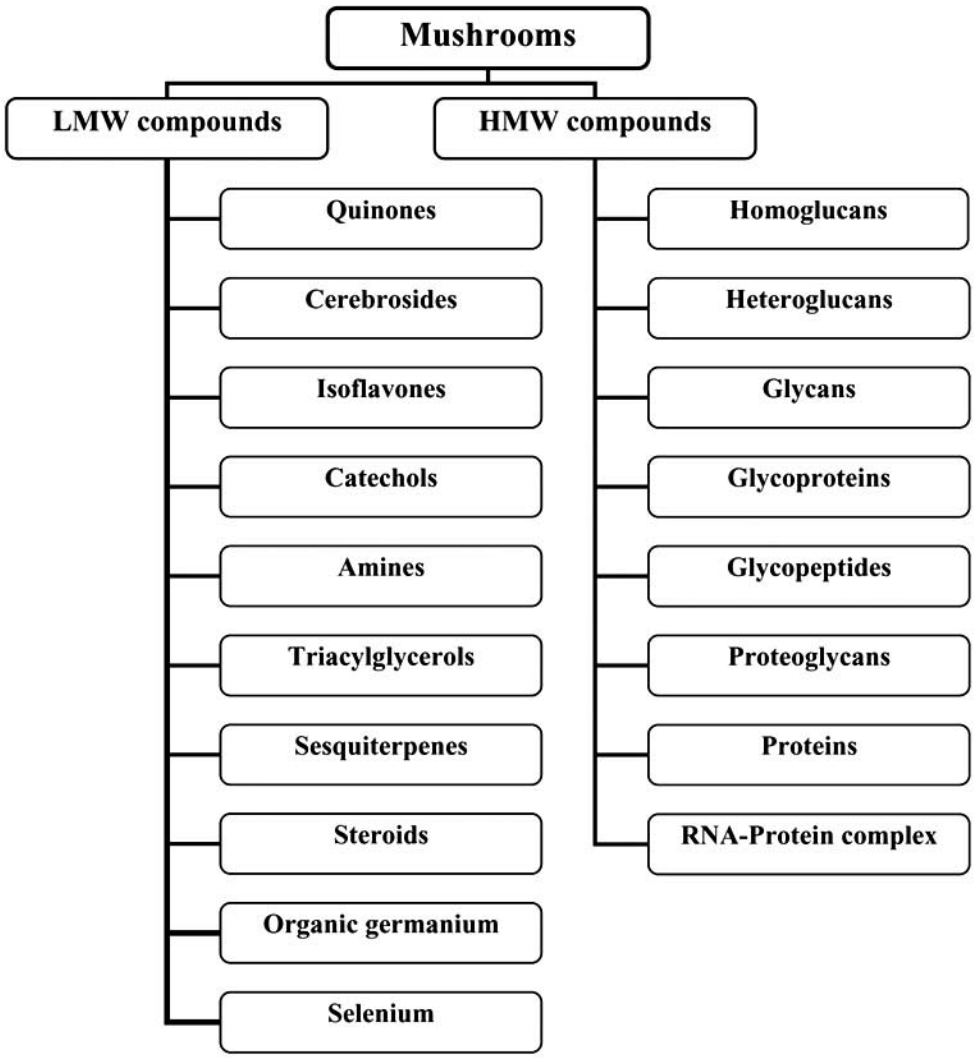


Fig. (1). Low-molecular-weight (LMW) and high-molecular-weight (HMW) compounds with antitumor potential found in mushrooms.

A potent sulfhydryl reagent appears in the gill tissues of the mushroom *Agaricus bisporus* in the period prior to sporulation. This agent, termed the 490 quinone (**4**) because of its maximum wavelength at 490 nm, has been postulated to play a role in the induction of cryptobiosis in the spore though inhibition of energy and DNA synthesis [36]. This compound markedly inhibited L1210 murine leukemia DNA polymerase α. The inhibition of DNA synthesis by the 490 quinone may contribute significantly to the cytotoxicity of this compound [12].

Two compounds from *Ganoderma lucidum* that inhibit eukaryotic DNA polymerase were identified as cerebrosides: (4*E*,8*E*)-N-D-2′-hydroxypalmitoyl-1-O-β-D-glucopyranosyl-9-methyl-4,8-sphingadienine (**6a**) and (4*E*,8*E*)-N-D-2′-hydroxystearoyl-1-O-β-D-glucopyranosyl-9-methyl-4,8-sphingadienine (**6b**). These cerebrosides selectively inhibited the activities of replicative DNA polymerases, especially the α-type, from phylogenetically broad eukaryotic species, whereas they had little effect on the activities of DNA polymerase β, prokaryotic DNA polymerases, terminal deoxynucleotidyl transferase, HIV reverse transcriptase, RNA polymerase, deoxyribonuclease I and ATPase. The inhibition of another replicative polymerase, the δ-type, was moderate [12,33].

The hydroquinone (*E*)-2-(4-hydroxy-3-methyl-2-butenyl)-hydroquinone (**5**) and polyporenic acid C (**24**) were isolated as matrix metallo-proteinase (MMPs) inhibitors, from the mushroom *Piptoropus betulinus*. This mushroom has been traditionally used in Czech Republic as a functional food for the treatment of rectal cancer [19]. Compound **24** was also isolated from *Daedalea dickinsii*. MMPs mediate physiological extracellular matrix remodelling and have been implicated in several pathological processes including tumor growth and metastasis [37].

In human hepatocellular carcinoma cells (HepG2), the isoflavone genistein (**7**) was found to modulate Cdc2 kinase activity and lead to G2/M arrest [38]. Furthermore, this compound suppressed the proliferation of p53-null human prostate carcinoma cells. The inhibitory effects of genistein on cell growth and proliferation were associated with a G2/M arrest in cell cycle progression, concomitant with a marked inhibition of cyclin B1 and an induction of Cdk inhibitor p21WAF1/CIP1 in a p53-independent manner [39].

The naturally occurring 6-(3,4-dihydroxystyryl)-4-hydroxy-2-pyrone (hispidin) (**8**) was isolated from the culture broth of *Phellinus linteus*, *Gymnopilus marginatus*, *G. patriae*, *G. parvisporus* and *Inonotus hispidus*. Hispidin is a potent inhibitor of PKCβ, a protein kinase which plays an important role in angiogenesis [12]. Hispidin synthesised by Gonindard *et al*. [40] was shown to be cytotoxic toward human keratinocytes (SLC-1 tumour cell line) and human pancreatic duct cells (Capan-1 tumour cell line). More importantly, the addition of hispidin in three successive doses led to a 100-fold increase in activity with an enhanced activity on cancer cells compared to normal cells.

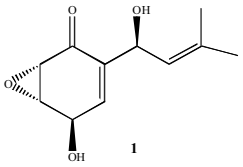
Table 1. Low-Molecular-Weight Compounds with Antitumor Potential

Class	Antitumor Agents	Mushroom Species	Molecular Targets	References
Quinones	Panepoxydone (1)	*Panus conchatus, P. rudis, Lentinus crinitus*	NF-KB inhibitor	[12]
	Cycloepoxydon (2)	*Xylaria* strain 45-93	NF-KB inhibitor	[12]
	Clavilactones CB (3a), CD (3b) and CA (3c)	*Clitocybe clavipes*	Tyrosine kinase inhibitors	[12,34,35]
	490 Quinone (γ-L-glutaminyl-4-hydroxy-2,5-benzoquinone) (4)	*Agaricus bisporus*	DNA polymerase α inhibitor	[12,36]
	(E)-2-(4-hydroxy-3-methyl-2-butenyl)-hydroquinone (5)	*Piptoporus betulinus*	MMPs inhibitor	[12,19]
Cerebrosides	(4E,8E)-*N*-D-2'-hydroxypalmitoyl-1-*O*-β-D-glucopyranosyl-9-methyl-4,8-sphingadienine (6a)	*Ganoderma lucidum*	DNA polymerase α inhibitors	[12,33]
	(4E,8E)-*N*-D-2'-hydroxystearoyl-1-*O*-β-D-glucopyranosyl-9-methyl-4,8-sphingadienine (6b)			
Isoflavones	Genistein (7)	*Flammulina velupites*	Cdc2 kinase modulator	[12,38,39]
Catechols	6-(3,4-dihydroxystyryl)-4-hydroxy-2-pyrone (Hispidin) (8)	*P. linteus, Gymnopilus marginatus, G. patriae, G. parvisporus, Ionotus hispidus*	PKCβ inhibitor	[12,40]
	Gerronemins A-F (9a-f)	*Gerronema*	COX-2 inhibitors	[12,43]
Amines	2-aminophenoxazin-3-one (10)	*Lepiota americana*	Aromatase inhibitor	[12,41]
	Putrescine-1,4-dicinnamide (11)	*Pholiota spumosa*	Not known (inducer of apoptosis and necrosis)	[13,47]
Triacylglycerols	1-Oleoyl-2-linoleoyl-3-palmitoylglycerol (12)	*Grifola frondosa*	Cyclooxygenase inhibitor	[12,44]
Sesquiterpenes	Iludin S and M (13a,b) and derivatives	*Omphalotus illudens, Lampteromyces japonicus*	One drivative is DNA-alkylating agents	[12,13]
Steroids	5,8-Epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol (14a)	*Lepiota americana, Cordyceps sinensis*	Sulfatase inhibitor	[12,41]
	5,8-Epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-D-glucopyranoside (14b) 5,6-Epoxy-24(*R*)-methylcholesta-7,22-dien-3β-ol (14c)	*Cordyceps sinensis*	Not known	[42]
	Ergosterol (15)	*Grifola frondosa, Agaricus blazei*	Cyclooxygenase inhibitor	[12,44,46]
	Ergosta-4,6,8(14),22-tetraen-3-one (16)	*Grifola frondosa, Ganoderma applanatum, G. neo-japonicum*	Cyclooxygenase inhibitor	[12,44,45]
	Lucidenic acid O (17a) Lucidenic lactone (17b) Cerevisterol (18) Lucidumol A (19) and B (20a) Ganoderiol F (20b) Ganodermanondiol (20c) Ganodermanontriol (20d) Ganoderic acids A (21a), F (21b), H (21c), W (22), X (23a), Y (23b), T (23c)	*Ganoderma lucidum*	DNA polymerase α, β and RT inhibitors (17a and 17b) DNA polymerase α inhibitor (18) NF-KB and AP-1 inhibitors (21a and 21c) DNA topoisomerase inhibitor (23a)	[12,33,48-52]
	Polyporenic acid C (24)	*Piptoporus betulinus, Daedalea dickinsii*	MMPs inhibitor	[12,19]
	Dehydroebriconic acid (25)	*Poria cocos*	DNA topoisomerase II inhibitor	[12]
	Fomitellic acids A and B (26a,b)	*Fomitella fraxinea*	DNA polymerase α and β inhibitors	[12]
Organic germanium	Bis-β-carboxyethylgermanium sesquioxide: O3(GeCH2CH2COOH)2	*Ganoderma lucidum*	Not known	[48,53]
Trace elements	Selenium	*Agaricus bisporus, Boletus edulis, Flammulina velutipes, Pleurotus ostreatus*	DNA cytosine methyltransferase inhibitor	[12]

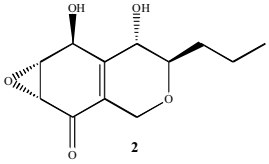
Sulfation and desulfation are important reactions in the metabolism of many steroid hormones. Aromatase inhibitors are used widely as second-line therapy in breast cancer and there is now evidence of a chemopreventive role for these agents [12]. Aromatase and sulfatase inhibitors were isolated from an edible mushroom, *Lepiota americana*. 2-Aminophenoxazin-3-one (**10**) inhibited aromatase, and 5,8-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-ol (**14a**) inhibited sulfatase [41].

Compound **14a** and two more steroids 5,8-epidioxy-24(*R*)-methylcholesta-6,22-dien-3β-D-glucopyranoside (**14b**) and 5,6-epoxy-24(*R*)-methylcholesta-7,22-dien-3β-ol (**14c**) isolated from *Cordyceps sinensis* inhibited the proliferation of K562, Jurkat, WM-1341, HL-60 and RPMI-8226 tumor cell lines [42].

Quinones



1



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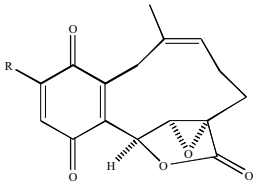
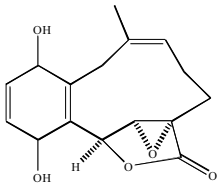
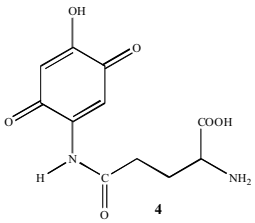


3a R=H; **3b** R=OH



3c



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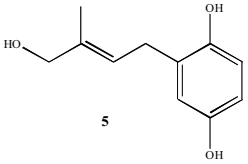

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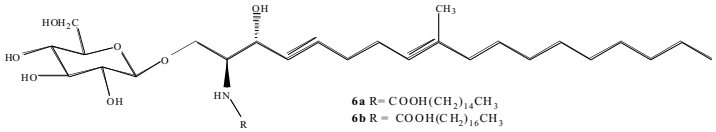
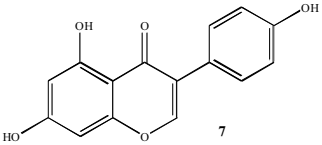
Cerebrosides



6a R= $COOH(CH_2)_{14}CH_3$
6b R = $COOH(CH_2)_{16}CH_3$

Isoflavones



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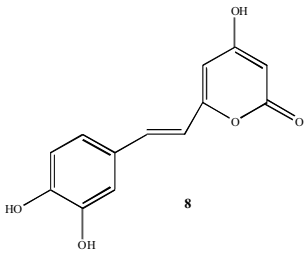
Catechols



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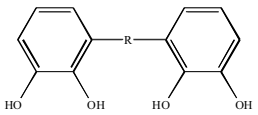
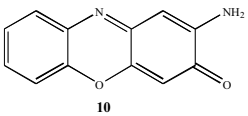


9a R= $(CH_2)_{12}$
9b R= $(CH_2)_3CH{=}CH(CH_2)_7$
9c R= $(CH_2)_{14}$
9d R= $(CH_2)_5CH{=}CH(CH_2)_7$
9e R= $CH_2CH{=}CH(CH_2)CH{=}CH(CH_2)_7$
9f R= $(CH_2)_4CH{=}CHCH_2CH{=}CH(CH_2)_7$

Amines



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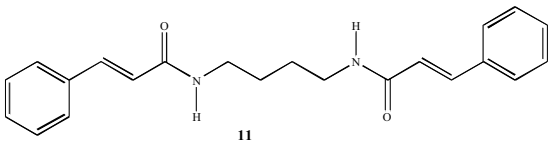

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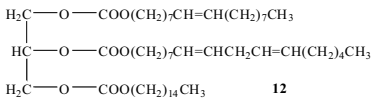
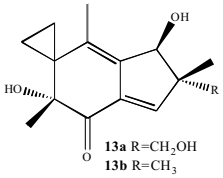
Triacylgycerols



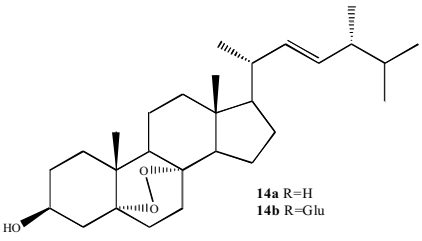
12

Sesquiterpenes

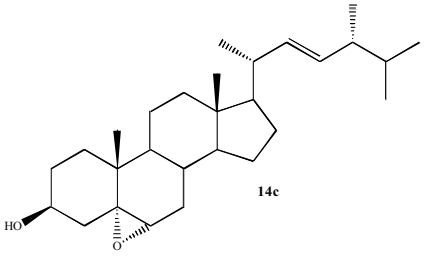


13a R=CH_2OH
13b R=CH_3

Steroids



14a R=H
14b R=Glu



14c

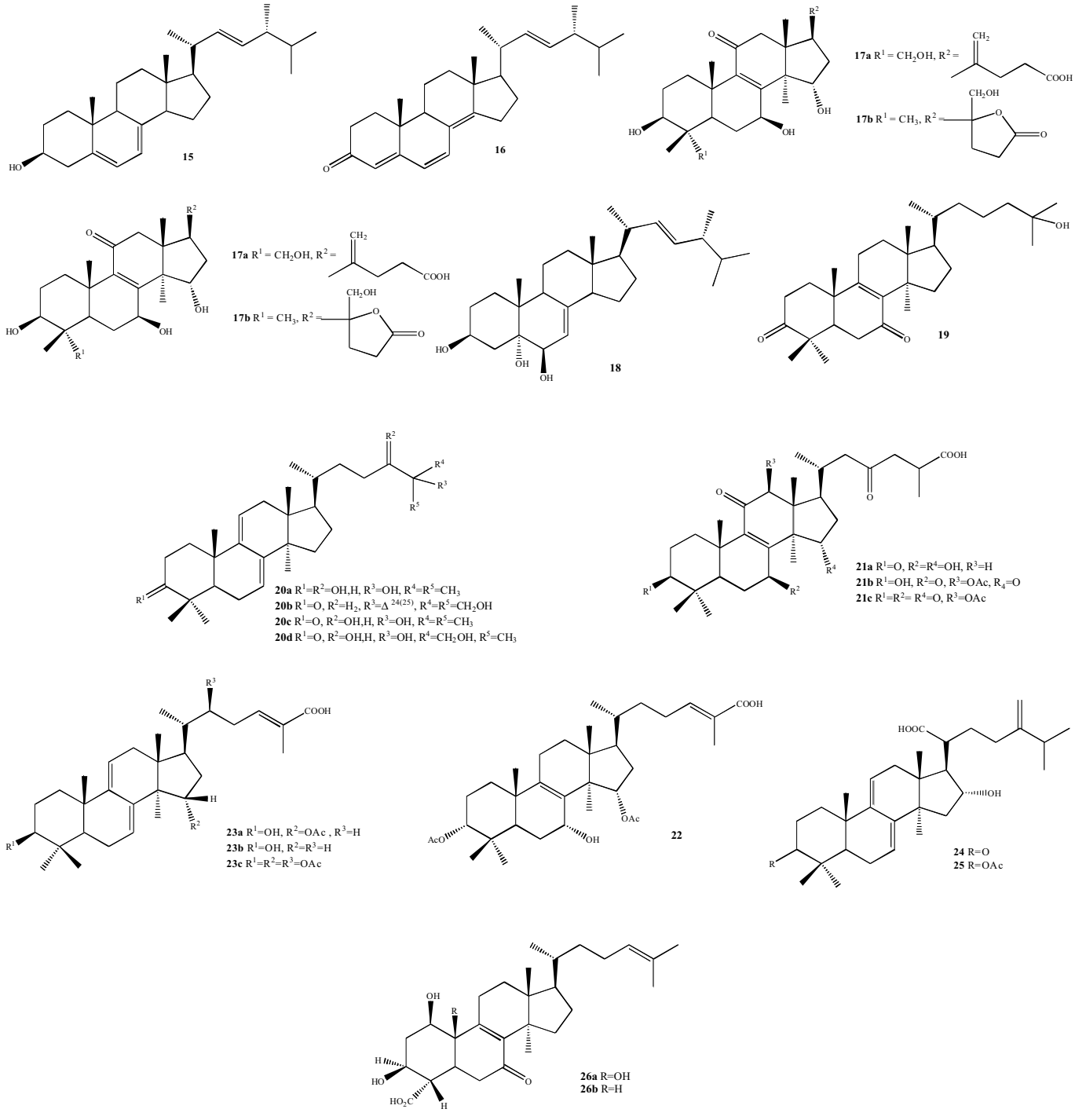


Fig. (2). Chemical structure of the low-molecular-weight (LMW) compounds with antitumor potential found in mushrooms.

It is now well established that the inducible isoform of cyclooxygenase, COX-2, is commonly over-expressed in many solid tumors. Epidemiological studies and clinical trials employing selective and nonselective COX-2 inhibitors indicate that COX-2 is mechanistically involved in colorectal carcinogenesis, gastric carcinoma, breast cancer, and prostate cancer [12]. The gerronemins A-F (**9**) blocked the inducible expression of a hCOX-2 and iNOS promoter driven reporter gene. These compounds were isolated as the cytotoxic components of an extract from a Gerronema species, found in the process of screening for new cytotoxic metabolites from basidiomycetes. Their structures were elucidated by spectroscopic techniques, and are composed of a C_{12}-C_{16} alkane or alkene substituted at both ends by 2,3-dihydroxyphenyl groups [43].

Other inhibitors of COX-1 and COX-2 enzymes are the compounds **15** ergosterol, **16** ergosta-4,6,8(14),22-tetraen-3-one and **12** 1-oleoyl-2-linoleoyl-3-palmitoylglicerol and were isolated from *Grifola frondosa* [12,44]. Compound **16** was also isolated from *Ganoderma applanatum* and G. *neo-japonicum* [45]. Compound **15** (ergosterol) was additionally isolated from the lipid fraction of *Agaricus brasiliensis* and retarded tumor growth in Sarcoma 180-bearing mice. In studies *in vivo* ergosterol inhibited neovascularisation, being a possible inhibitor of angiogenesis [12,46].

Pholiota spumosa produces a polyamine (putrescine-1,4-dicinnamide) (**11**) that inhibits the growth of human prostate cancer cell lines, inducing apoptotic cell death. This is mediated, at least partly, by the activation of caspase cascades. At higher doses the mode of cell death was described as necrosis [13,47].

Omphalotus illudens and *Lampteromyces japonicus* produce compounds with potential antitumor properties, as sesquiterpenes illudin S (**13a**) and illudin M (**13b**). Because of its high toxicity, illudin S was not studied further and hemisynthetic derivatives were produced as irofulven, a compound that demonstrates possible anticancer properties against solid tumors, by working as a DNA-alkylating agent. In addition, its cytotoxicity seems to be more specifically addressed against malignant cells, having shown less selectivity for normal cells [13]. Irofulven has demonstrated antitumor potential against various human cancer cell lines such as pancreatic carcinoma, colorectal carcinoma, ovarian carcinoma, non-small-cell lung cancer and malignant glioma, amongst others [12].

Ganoderma lucidum is a mushroom with many interesting compounds [13,33]. These include polysaccharides of β-glucan type and proteins such as LZ-8, as it will be discussed in the next section, but also steroids **17-23**. The described antitumor potential of such compounds is similar to that described for β-D-glucans: activators of the NF-kB pathway and modulators of Ras/Erk, c-myc, CREB protein and mitogen-activated protein kinases [12,48]. Lucidenic acid O (**17a**) and lucidenic lactone (**17b**) prevented not only the activities of calf DNA polymerase α and rat DNA polymerase β, but also those of human immunodeficiency virus type 1 reverse transcriptase. Cerevisterol (**18**), which was reported to be a cytotoxic steroid, inhibited only the activity of DNA polymerase α [49].

Ganoderic acids W (**22**), X (**23a**) and Y (**23b**) and lanostane-type triterpenes isolated from spores of *Ganoderma lucidum*, the ganoderic alcohols lucidumol A (**19**) ((24S)-24, 25-dihydroxylanost-8-ene-3, 7-dione) and lucidumol B (**20a**) (β,(24S)-lanosta-7, 9(11)-diene-3β, 24, 25-triol), ganodermanondiol (**20c**), ganoderiol F (**20b**) and ganodermanontriol (**20d**) have been shown to exert a cytotoxic effect in some tumor cell lines [48]. Ganoderic acid X (**23a**) and ganoderic acid T (**23c**) induced apoptosis in human hepatoma and lung cancer cells, respectively, suggesting that the basic lanostane structure is necessary for the biological activity of purified triterpenes [50]. Treatment of human hepatoma HuH-7 cells with ganodermic acid X (**23a**) caused immediate inhibition of DNA synthesis as well as activation of ERK and JNK nitrogen-activated protein kinases and cell apoptosis. The apoptotic molecular events were elucidated and included degradation of chromosomal DNA, a decrease in the level of Bc1-xL, disruption of mitochondrial membrane, release of cytocrome c into the cytosol and activation of caspase-3. The ability of compound **23a** to inhibit topoisomerases and to sensitize cancer cells to apoptosis makes it an interesting compound in the context of this review [51]. Animal experiments *in vivo* showed that compound **23c** suppressed the growth of human solid tumors xenographed in athymic mice. It was also shown to markedly inhibit the proliferation of a highly metastatic lung cancer cell line (95-D), by apoptosis induction (with reduction of mitochondria membrane potential and release of cytochrome c) and cell cycle arrest at G1 phase [52].

Ganoderic acid A (**21a**) and H (**21c**) suppressed the growth of MDA-MB-231 cells (highly invasive human breast cancer cells), confirmed by assays of cell proliferation and colony formation, together with assays of invasive behaviour (adhesion, migration and invasion). The compounds mediate their biological effects through the inhibition of the transcription factors AP-1 and NF-kappaB, resulting in the down-regulation of expression of Cdk4 and the suppression of secretion of urokinase-type plasminogen activator (uPA), respectively. Furthermore, the activity of ganoderic acids is linked to the hydroxylation in the position 7 and 15 (compound **23a**) and 3 (compound **23c**) in their triterpene lanostane structure.

Dehydroebriconic acid (**25**) was found in sclerotia of *Poria cocos*. This compound potently inhibited DNA topo II activity, while moderately inhibited the activities of DNA polymerases α, β, γ, δ, ε, η, ι, κ, and λ from mammals, to similar extents. Fomitellic acids A and B (**26**) from the basidiomycete *Fomitella fraxinea*, have selectively inhibited the activities of mammalian DNA polymerases α and β [12].

The germanium content of Ganoderma extract is interesting because Ganoderma extract is claimed, like ginseng, to contain a comparatively high content of germanium (Ge), and this metal has been associated with immunomodulatory, antioxidant and antitumor effects [48,53].

The recognition that selenium has anticancer properties, with the ability of mushrooms to accumulate this element, has created a market niche for selenium-enriched mushrooms (e.g., *Agaricus bisporus*, *Flammulina velutipes*, *Pleurotus ostreatus*). Several mechanisms (including DNA cytosine methyltransferase inhibition, antioxidant protection, enhanced immune surveillance, and inhibition of angiogenesis) have been proposed to account for the anticancer potential of selenium. However, the selective induction of apoptosis in tumor cells may be of special significance [12].

3.2. High Molecular Weight Compounds with Antitumor Potential

Many, if not all, Basidiomycete mushrooms contain biologically active compounds in fruit bodies, cultured mycelium, or culture broth [8]. Tables **2** and **3** list the type and source of some mushrooms' high-molecular weight compounds with demonstrated antitumor potential. Among these, some polysaccharides and polysaccharide conjugates have been approved in some countries for the clinical treatment of cancer patients. They are "Lentinan" from the fruiting bodies of Shiitake (*Lentinus edodes*), "Schizophyllan" (Sonifilan, SPG) from the culture fluid of Suehirotake (*Schizophyllum commune*), and "Krestin" (PSK), from the cultured mycelium of Kawaratake (*Trametes versicolor*). Lentinan and schizophyllan are pure β-glucans, whereas PSK is a protein bound polysaccharide. The biological activity of these three products is related to their immunomodulating properties, which enhance the person's own defence against various forms of infectious disease. These immunopotentiators, or immunoinitiators, are also referred to as "biological response modifiers" [12,54-56]. In fact, available evidence indicated that the antitumor properties of β-glucans were attributable to enhancement of the numbers and/or functions of macrophages, natural killer (NK) cells, and subsets of T cells, that is, the modulation of both innate and adaptive immunity [21]. Calvacin is a potent antitumor mucoprotein isolated in the 1960s from the giant puffball (*Calvatia gigantean*) fruiting body which causes a prolonged intoxication characterized by anorexia and extreme weight loss [57,58].

The natural polysaccharides isolated from mushrooms, described as having antitumor potential, include different types of glicosidic linkages (Table **2**), while some are bound to protein or peptide residues such as polysaccharide-protein or -peptide complexes (Table **3**) [13,21,25,56,59]. Data on mushroom polysaccharides, with most belonging to the group of β-glucans, has been collected from more than 650 species representing 182 genera of higher Basidiomycetes [8]. Potential antitumor activity has been described by a wide range of glycans, extending from homopolymers to highly complex heteropolymers (Table **2**). Polysaccharides with antitumor potential from various mushrooms are characterized by their molecular weight, degree of branching, and higher (tertiary) structure. Homoglucans are linear or branched molecules having a backbone composed of α- or β-linked glucose units (such as (1→3), (1→6)-β-glucans and (1→3)-α-glucans), and some contain side chains that are attached at different positions. Among them, β-glucans are the most important polysaccharides with antitumor potential. These are glucose polymers that can exist

Table 2. High-Molecular-Weight Compounds with Antitumor Potential: Polysaccharides

Class	Antitumor Agents	Mushroom Species	Details	References
Homoglu-cans	Linear (1→3)-β-D-glucan	*Auricularia auricula* *Lyophyllum decastes*	Immunomodulator; pre-clinical animal models	[56]
	(1→3)-β-D-glucan with (1→6)-β-D branches: Lentinan	*Lentinus edodes*	Immuno-enhancing activity; clinical trials (gastric, colorectal, prostate and breast cancers)	[8,12,13,19,48,56,60-63]
	Schizophyllan, SPG	*Schizophyllum commune*	Immuno-enhancing activity; clinical trials (gastric, cervical, head and neck cancers)	[8,12,19,21,48,56]
	Grifolan, GRN	*Grifola frondosa*	Immuno-enhancing activity; clinical trials (gastro-intestinal, lung, liver and breast cancers)	[12,13,19,56]
	Scleroglucan, SSG	*Sclerotinia sclerotiorum*	Immunomodulator; clinical trials	[48]
	Alkali-soluble glucan	*Pleurotus tuber-regium*	Immunomodulator; *in vitro* cell lines	[19]
	Pleuran	*Pleurotus ostreatus*	Immunomodulator; pre-clinical animal models	[64]
	SCG	*Sparassis crispa*	Immunomodulator; pre-clinical animal models	[19,48]
	H-3-B	*Cryptoporus volvatus*	Immunomodulator; pre-clinical animal models	[19]
	PG101	*Lentinus lepideus*	Immunomodulator; pre-clinical animal models	[48]
	GLP	*Ganoderma lucidum*	Immunomodulator; pre-clinical animal models	[48]
	(1→3)- β-D-glucan with (1→2) or (1→6) branches	*Pachyman* from *Poria cocos*	Immunomodulator; pre-clinical animal models	[56]
	Linear (1→6)- β-D-glucan	*Armillariella tabescens* *Lyophyllum decastes*	Immunomodulator; pre-clinical animal models	[8,56]
	(1→6)-β-D-glucan with (1→3)-β-D branches: D-fraction	*Agaricus blazei* *Grifola frondosa*	Immunomodulator; clinical trials (breast, prostate, lung, liver, and gastric cancers)	[12,48,65]
	(1→6)-β-D-glucan with (1→4)- α branches	*Agaricus blazei*	Immunomodulator; pre-clinical animal models	[56]
	(1→3)-α- glucan	*Amanita muscaria* *Agrocybe aegerita* *Agaricus blazei* *Armillariella tabescens*	Immunomodulator; pre-clinical animal models	[8,56]
	(1→3)-α–glucan with (1→6)-β branches	*Agaricus blazei*	Immunomodulator; pre-clinical animal models	[56]
	(1→6)-α–glucan with (1→4)-α branches	*Agaricus blazei*	Immunomodulator; pre-clinical animal models	[8]
Heteroglu-cans	Arabinoglucan	*Ganoderma tsugae*	Immunomodulator; pre-clinical animal models	[8,48,56]
	Galactomannoglucan	*Flammulina velutipes,* *Hohenbuehelia serotina,* *Leucopaxillus giganteus*	Immunomodulator; pre-clinical animal models	[8,48,56]
	Galactoxyloglucan	*Hericium erinaceus*	Immunomodulator; *in vitro* cell lines	[8,48,56]
	Mannogalactoglucan	*Agaricus blazei* *Ganoderma lucidum* *Pleurotus pulmonariu* *Pleurotus cornucopiae*	Immunomodulator; *in vitro* cell lines	[8,48]
	Mannoxyloglucan	*Grifola frondosa*	Immunomodulator; pre-clinical animal models	[8,48,56]
	Riboglucan	*Agaricus blazei* *Flammulina velutipes*	Immunomodulator; *in vitro* cell lines	[8,48,56]
	Xyloglucan	*Agaricus blazei* *Grifola frondosa* *Polyporus confluens* *Pleurotus pulmonarius*	Immunomodulator; pre-clinical animal models	[8,48,56,66]
	Xylogalactoglucan	*Inonotus obluquus*	Immunomodulator; *in vitro* cell lines	[8,48,56]
	(1→3)-β-glucuronoglucan	*Ganoderma lucidum*	Immunomodulator; *in vitro* cell lines	[48,56]
Glycans	Arabinogalactan	*Pleurotus citrinopileatus*	Immunomodulator; pre-clinical animal models	[8,56]
	Fucogalactan	*Sarcodon aspratus*	Immunomodulator; pre-clinical animal models	[8,56]
	Fucomannogalactan	*Dictyophora indusiata* *Grifola frondosa*	Immunomodulator; pre-clinical animal models	[8,56]
	Glucogalactan	*Ganoderma tsugae*	Immunomodulator; pre-clinical animal models	[8,48,56]
	Mannogalactan	*Pleurotus pulmonarius*	Immunomodulator; *in vitro* cell lines	[8,48,56]
	Mannofucogalactan	*Fomitella fraxinea*	Immunomodulator; *in vitro* cell lines	[8]
	Xylan	*Hericium erinaceus*	Immunomodulator; pre-clinical animal models	[8,48,56]
	Glucoxylan	*Hericium erinaceum* *Pleurotus pulmonarius*	Immunomodulator; pre-clinical animal models	[8,48,56]

(Table 2) contd….

Class	Antitumor Agents	Mushroom Species	Details	References
	Mannoglucoxylan	*Hericium erinaceus*	Immunomodulator; pre-clinical animal models	[8,48,56]
	Mannogalactofucan	*Grifola frondosa*	Immunomodulator; pre-clinical animal models	[8,48,56]
	Mannan	*Dictyophora indusiata*	Immunomodulator; pre-clinical animal models	[8,56]
	Glucomannan	*Agaricus blazei*	Immunomodulator; pre-clinical animal models	[8,48,56]
	Galactoglucomannan	*Lentinus edodes*	Immunomodulator; pre-clinical animal models	[48,56]
	Galactomannan	*Morchella esculenta*	Immunomodulator; pre-clinical animal models	[56]
	Tremellastin (Glucuronoxylomannans)	*Tremella fuciformis*	Immunomodulator; pre-clinical animal models	[19]

Table 3. High-Molecular-Weight Compounds with Antitumor Potential: Glycoproteins and Proteins

Class	Antitumor Agents	Mushroom Species	Details	References
Glycoproteins (Polysaccharide-protein complexes)	ATOM	*Agaricus blazei*	Immunomodulator; pre-clinical animal models	[48]
	AB-FP	*Agaricus blazei*	Immunomodulator; clinical trials	[8,66]
	PSPC	*Tricholoma lobayense*	Immunomodulator; pre-clinical animal models	[48,67]
	Galactoxyloglucan-protein complex	*Hericinum erinaceus*	Immunomodulator; *in vitro* cell lines	[19]
	Glucoxylan-protein complex	*Hericium caput-medusae*	Immunomodulator; *in vitro* cell lines	[19]
	Ganoderans	*Ganoderma lucidum*	Immunomodulator; *in vitro* cell lines	[19]
Glycopeptides (Polysaccharide-peptide complexes)	PSP	*Trametes versicolor*	Immuno-enhancing activity; clinical trials	[12,13,19,31,48,56,68]
	PSK or krestin	*Trametes versicolor*	Immuno-enhancing activity; clinical trials (head, neck, upper gastro-intestinal, colorectal, lung , and breast cancers)	[8,12,13,19,31,48,68]
	KS-2	*Lentinus edodes*	Immunomodulator; *in vitro* cell lines	[19,60]
	Glycopeptide complexes	*Ganoderma lucidum*	Immunomodulator; pre-clinical animal models	[33,48]
Proteoglycans	GLIS	*Ganoderma lucidum*	Immunomodulator; pre-clinical animal models	[48]
	PL	*Phellinus linteus*	Immunomodulator; pre-clinical animal models	[48,69]
Mixture of polysaccharides and lignin	LEM	*Lentinus edodes*	Immunomodulator; pre-clinical animal models	[19,60]
Proteins	Flammulin	*Flammulina velutipes*	Immunomodulator; *in vitro* cell lines	[19,70]
	Protein LZ8	*Ganoderma lucidum*	Immunomodulator; pre-clinical animal models	[19,48,71]
	Clitocypin	*Clitocybe nebularis*	Cysteine proteinase inhibitor; *in vitro* enzymatic inhibition assays	[72,73]
	Lectins	*Agaricus bisporus, Boletus satanas, Grifola frondosa, Tricholoma mongolicum, Volvariella volvacea*	Immunomodulator and antiproliferative; *in vitro* cell lines	[71]
RNA-protein complex	FA-2-b-Md	*Agaricus blazei*	Immunomodulator; *in vitro* cell lines	[19]

as a non-branched (1→3)-β-linked backbone or as a (1→3)-β-linked backbone with (1→6)-β-branches, occurring as a primary component in the cell walls of higher fungi in great amounts [48]. It was described that β-(1→3) linkages in the main chain of the glucan and additional β-(1→6) branch points are needed for antitumor properties. The β-glucans containing mainly (1→6) linkages have less activity. Besides the degree of branching, molecular weight, number of substituents, as well as ultrastructure, including the presence of single and triple helices, significantly affect the biological activities of β-glucans. Higher antitumor potential seems to be correlated with higher molecular weight [8], lower level of branching and greater water solubility of β-glucans. However, the high branched MD-fraction from *G. frondosa* has been shown to have high antitumor potential [19].

Lentinan is a high molecular weight (about one million) homopolysaccharide in a triple helix structure, with linear chains consisting of (1-3)-β-Dglucopyranosyl (Glcp) residues with two β-(1-6)-linked Glcp branchings for every five β-(1-3)-Glcp residues [60]. This homoglucan from *L. edodes* has been studied in pre-clinical animal models and human clinical practice, and has been described as simultaneously demonstrating antitumor intrinsic activity and prophylactic ability. This compound is currently approved for the treatment of gastric cancer in Japan. Lentinan administered directly into tumors decreased the ratio of suppressor–inducer T cells and suppressor T cells tended to increase the ratio of cytotoxic T cells (CTLs) and IL-2 production of lymph node lymphocyte [61]. It has been shown to augment the activities of NK cells, lymphokine-activated killer cells and CTLs. It can also activate macrophage differentiation and increases response in delayed-type hypersensitivity against tumor antigen [48]. The single inconvenience comes from poor oral absorption, requiring administration by injection. Nonetheless, this molecule is of interest as adjunct therapy in cancer to induce cytostatic effects [13]. In clinical studies for anticancer properties, lentinan has been described as prolonging the overall survival of cancer patients, especially those with gastric and colorectal carcinomas, metastatic prostate and breast cancer [62,63].

SPG from *Schizophyllum commune* showed antitumor activity against both the solid and ascite forms of Sarcoma 180, as well as against the solid form only of sarcoma 37, Erlich sarcoma, Yoshida sarcoma and Lewis lung carcinoma [8]. It has also increased cellular immunity by restoring suppressed killer-cell activity to normal levels in mice with tumors [21]. Human clinical studies claimed the benefit of treating with schizophyllan patients presenting recurrent and inoperable gastric cancer, stage 2 cervical cancer, and advanced cervical carcinoma [8]. SPG has also been shown to increase overall survival of patients with head and neck cancers [48]. Additionally, it is also used as assistant immunotherapy in the treatment of gastric cancer [12].

Grifolan from *Grifola frondosa* showed antitumor potential in gastrointestinal, lung, liver and breast cancers [13]. This compound is a macrophage activator which augments cytokine production without dependence on endotoxins. Additionally, it enhances the mRNA level of IL-6, IL-1 and TNF (tumor necrosis factor)-α of macrophages.

SSG, a homoglucan from *Sclerotinia sclerotiorum*, induces the development of TH1 cells via the IL-12 pathway. SCG from *Sparassis crispa* enhances the hematopoietic response [48].

Pleuran (β-1,3-D-glucan) isolated from the oyster mushroom *Pleurotus ostreatus* has been proven to decrease the development of precancerous aberrant crypt foci lesions in the colon, when studied in the male Wistar rat [64].

PG101 from *Lentinus lepideus* can recover the radiation-damaged bone marrow system very efficiently, increasing the levels of IL-1β, IL-6, granulocyte macrophage-colony stimulating factor (GM-CSF) and reducing the level of TNF-α [48].

The maitake D-fraction is a relatively new compound, and there are a number of clinical trials in breast, prostate, lung, liver, and gastric cancers underway in the United States and Japan. Most of these are at an early clinical stage (phase I/II) [65].

Heteroglucan side chains contain arabinose, galactose, mannose, ribose, xylose or glucuronic acid, and may have different combinations [8]. For example, a xyloglucan (Xyl:Glc, molar ratio = 2:10) containing 9% protein obtained by fractionation and purification of *Agaricus blazei* extract showed significant activity against Sarcoma 180 in mice [66]. Another large group of bioactive polysaccharides is called glycans, which contain units other than glucose in their backbone. They are classified as galactans, fucans, xylans, and mannans by the individual sugar components in the backbone [48]. Unlike β-(1→3)-glucans, α-(1→3)-glucuronoxylomannans, which are characteristic of jelly mushrooms, are not strongly dependent on molecular weight [8]. Galactomannan from *Morchella esculenta* enhances macrophage activation, increasing nuclear factor kappa B (NF-kB)-directed luciferase expression in THP-1 human monocytic cells. Fucogalactan, from *Sarcodon aspratus,* increase the release of TNF-α and NO in macrophages of mice *in vitro* [48]. β-(1→2)-; β-(1→3)-Glucomannan is a polysaccharide active against Sarcoma 180, separated from the liquid cultured mycelium of *Agaricus blazei* [8].

In some mushroom species, polysaccharides are covalently bound to proteins or peptides as polysaccharide-protein or -peptide complexes which also showed antitumor potential (Table **3**). Glycoproteins are polysaccharide-protein complexes and such compounds in mushrooms include β-glucan-protein, α-glucan-protein and heteroglycan-protein complexes. An example is a polysaccharide-protein complex (PSPC) isolated from the culture filtrates of the edible mushroom, *Tricholoma lobayense,* by ethanol precipitation. PSPC contained 40% polysaccharide which consisted of galactose, glucose, mannose, fucose, arabinose and rhamnose, and 30.05% protein which was composed of 15 amino acids including aspartic, glutamic and other acidic amino acids. This glycoprotein could restore and increase phagocytic function of macrophages of the tumor-bering mice [48,67]. Another example is ATOM from *Agaricus blazei*. It has been suggested that the tumor growth inhibitory effect of ATOM is apparently due to immunological host-mediated mechanisms so that it is caused by increasing the number of peritoneal macrophages, the phagocytosis of polystyrene latex beads and the proportion of the third component of complement (C3)-positive fluorescent cells in the tumor-bearing mice [48]. A liquid medium filtrate separated from the mycelium after submerged cultivation of *A. blazei* contained mannan-protein complex (AB-FP) with a molecular weight of 105–107 Da and a small amount of glucose, galactose, and ribose. The yield of AB-FP was 575 mg/l liquid medium filtrate, and it was described as possessing significant antitumor activity after animal studies and in clinical experience [8,66].

Glycopeptides are a group structurally similar to glycoproteins but with a smaller chain of amino acids. PSP and PSK are examples of this class of compounds with antitumor properties. Both products are obtained from the extraction of *Trametes versicolor* mycelia. PSK and PSP are Japanese and Chinese products, respectively. Both products have similar physiological activities but are structurally different. PSK and PSP are produced from CM-101 and Cov-1 strains of *T. versicolor*, respectively. Both products are obtained by batch fermentation. PSK fermentation lasts up to 10 days, whereas PSP production involves a 64h culture. PSK is recovered from hot water extracts of the biomass by salting out with ammonium sulfate, whereas PSP is recovered by alcoholic precipitation from the hot water extract [31]. PSK is 1,3 and 1,6-monoglucosyl branched 1,4-β-D-glucan binding to aspartic, glutamic and other acidic amino acids. PSP resembles the PSK structure but is richer in glutamic and aspartic acids [48]. The presence of fucose in PSK and of rhamnose and arabinose in PSP distinguishes the compounds [19]. There have been several decades of successful clinical trials using PSK to treat head and neck, upper gastro-intestinal, colorectal, and lung cancers with some reported success in treating breast cancer as well [68]. While PSK has been almost exclusively developed and tested in Japan, PSP, in contrast, is a product of China and continues to be assessed for efficacy safety by their scientists and oncologists. Many Phase III clinical trials of PSP combined with conventional therapies have demonstrated significant benefits against cancers of the stomach and lung [12]. Both compounds are of great interest as adjuvants of cancer chemotherapy and radiotherapy. They improve regular therapeutic efficacy and tolerance (reduction of side effects), slow down tumor growth and tend to prevent metastasis. On a general point of view, global patient health status is significantly improved in gastric, intestinal and lung cancer. The precise molecular mechanism of action of PSP and PSK is still not elucidated. Nonetheless, these compounds most likely act through the enhancement of the immune system (increasing the number of immune cells and facilitated CDs and cytotoxic T-cell infiltration of tumors) more than via direct cytotoxic effects [13,31,48]. KS-2 from *L. edodes* is another example of the peptide–polysaccharide complex with antitumor properties [60].

Proteoglycans are another class of glycoproteins but heavily glycosylated. They consist of a core protein with one or more coavalently attached glycosaminoglycan chain(s) [48]. An example is GLIS from *Ganoderma lucidum* and PL from *Phellinus linteus*. GLIS contains carbohydrates and protein in a ratio of 11.5:1 so that the carbohydrate portion is formed by seven different monosaccharides, predominantly D-glucose, D-galactose, and D-mannose in the molar ratio of 3:1:1. It is a B-cell stimulating factor [48]. The acidic proteoglycan (PL) was shown to be a (1,6) branched type (1,3) glycan containing a mixture of monosaccharide including mannose, glucose, arabinose and xylose with both α- and β-link-ages, and a variety of amino acids including aspartic acid, glutamic acid, alanine, glycine and serine [69]. PL can modulate circulating cytokine responses in lippolysaccharide (LPS)-treated mice and the *in vivo* administration of this compound decreased IL-2 and TNF-α production in splenocytes and enhanced cell apoptosis in macro-

phages and lymphocytes stimulated with LPS *in vitro*. The inhibitory effect of PL on the growth of MCA-120 tumor cells was associated with its immunoregulating properties including the induction of IL-12 and interferon (IFN)-γ production, leading to a TH1 dominant state [48]. LEM is a mycelial extract preparation of *L. edodes* harvested before the cap and stem grow, which also revealed antitumor potential [19]. It is a heteroglycan–protein conjugate containing 24.6% protein and 44% sugars, comprising mostly pentoses as well as glucose and smaller amounts of galactose, mannose and fructose. It also contains nucleic acid derivatives, B complex vitamins, ergosterol, eritadenine (an anticholesteremic amino acid) and water-soluble lignins [60].

Besides polysaccharides, several proteins identified in mushrooms have been described as having antitumor activity, including flammulin from *Flammulina velutipes*, protein LZ8 from *Ganoderma lucidum*, clitocypin from *Clitocybe nebularis*, and lectins from different species (Table **3**). The antitumor protein Flammulin comprises large amounts of aspartic acid, a higher number of arginine residues than of lysine residues, and no methionine [70]. LZ-8, a fungal immunomodulatory protein, is a potent T-cell activator, mediating its effects via cytokine regulation of integrin expression [48]. It has a sequence of 110 amino acid residues which is blocked at the *N*-terminus by *N*-acetylation and has less than 2% carbohydrate [71]. Clitocypin is a novel cysteine proteinase inhibitor and is the first to be characterized from higher fungi. Its sequence of 150 amino acid residues, M = 16.9 kDa, contains no cysteine or methionine [72,73]. Lectins are storage proteins exhibiting a diversity of chemical characteristics. Some of them are monomeric, whereas others are dimeric, trimeric or tetrameric. Their molecular weights range from 12 to 190 KDa, with sugar contents from 0 to 18%. Carbohydrate specificities involve mainly galactose, lactose and *N*-acetylgalactosamine. A small number of mushroom lectins are specific for fucose, raffinose, *N*-glycolyneuraminic acid and *N*-acetyl-D-lactosamine. Several lectins have been described as having potential antitumor/cytotoxic activities, particularly against the human colon cancer cell lines HT29 and Caco-2 and breast cancer cell line MCF-7 (lectins from *Agaricus bisporus*), HeLa cells (lectins from *Grifola frondosa*), mouse mastocytoma P815 cells (lectins from *Tricholoma mongolicum*) and sarcoma S-180 cells (lectins from *Volvariella volvacea*). Additionally, lectins from *Boletus satanas* induced the release of tumor necrosis factor-α from mononuclear cell cultures [71].

CONCLUDING REMARKS

It is estimated that 50% of the annual 5 million metric tons of cultivated mushrooms might contain functional or medicinal properties, which may be used as a source of biologically and physiologically active substances [32]. Therefore, it is very important the isolation, structural characterization and classification of compounds from mushrooms with potential antitumoral properties. Higher fungi may provide potent compounds with potential to be used in the prevention and treatment of cancer. However, most of the research here reviewed is relatively recent and has been based on tumor cell line or animal models. Only little work has been performed at the level of clinical trials in patients. Therefore, despite the promising published data, more work needs to be carried out to clarify the real use of mushrooms, or their isolated compounds, in the prevention and treatment of cancer.

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