ANTINEOPLASTIC POTENTIAL OF MYCOTOXINS

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Fungal toxins are secondary metabolites, in which many of them were mycotoxins, affecting eukaryotic cells with a broad range of structural and functional variety contributing to the multitude of their classification. This refers to the harmful genotoxic (mutagenic, teratogenic, and carcinogenic) effects of mycotoxins on the one hand, and their cytocidic and antineoplastic properties on the other hand. This "double edged sword" effect could be utilized against the spread of tumors in older patients when the survival is much more important than the mutagenic side effects. To decide which fungal toxins could be used as combined cytotoxic and antimetastatic agents, mycotoxins were divided into three categories: (a) highly genotoxic (mutagenic, teratogenic, and carcinogenic), (b) adversely toxic, and (c) antitumorigenic agents. Highly cytotoxic mycotoxins with tolerable side effects, combined with an antineoplastic character, could be potential candidates against metastasis. From the structure–function relationship of antimetastatic mycotoxins, only general conclusions have been drawn. The presence of ring structures containing heteroatoms, functional groups, and the cumulative presence of oxygen atoms contributed to the oxidative stress and cytotoxicity of mycotoxins. The preselection of mycotoxins excluded category (a), and only the categories (b) and (c) were considered to be potential agents against the metastatic spread of abdominal tumors in rodent metastatic tumor experiments.

Keywords: carcinogens, antitumor agents, structure–function relationship, metastatic connection

Introduction

Physicians distinguish among mycotoxins either depending on the affected organ (nephro-, hepato-, immunotoxins, etc.) or by the type of illness known as fungal toxicosis [1, 2]. Cell biologists try to separate the cytotoxic, mutagenic, teratogenic, and carcinogenic effects of mycotoxins [3, 4]. Chemists classify mycotoxins either on the basis of chemical structures (lactone, coumarin,

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sesquiterpene, ergot, and difuran) or on the biosynthetic origin (polyketide, peptide-like, etc.) [1, 5, 6]. Toxicological considerations suggested the selection of mycotoxins based on their (a) genotoxic, (b) cytotoxic, and (c) anticancer properties.

As the function of mycotoxins has not been clearly defined, it was assumed that the chemistry or biology could explain their adverse health effects [7]. This review is selecting among those mycotoxins that possess strong toxic effects against tumor cells with tolerable side effects. In conformity with this strategy in mind, the focus is placed on those mycotoxins or their precursors that besides being highly cytotoxic, attest antitumor properties and as potential basic compounds could be considered as antimetastatic agents in future animal experiments. Based on their adverse effects, mycotoxins were divided into three categories: (a) highly genotoxic (mutagenic, teratogenic, and carcinogenic), (b) adversely toxic (highly toxic, low, beneficially toxic, apoptotic, and necrotic), and (c) antitumorigenic agents. Carcinogenic compounds have been excluded from the selection, and only those compounds will be considered for future antimetastatic animal experiments that show combined cytotoxic and antineoplastic properties.

Genotoxic Mycotoxins

Almost certainly, the main human and veterinary health burden of mycotoxin harm is related to its chronic exposure leading among others to cancer induction, tissue toxicity, and immunosuppression [8]. Those mycotoxins that cause severe carcinogenic, mutagenic, teratogenic, and immunosuppressive effects are *ab ovo* excluded from the race of becoming antineoplastic agents. Figure 1 shows only structures of mycotoxins with strong toxic and anticancer potential to be considered for selection. Tumorigenic mycotoxins are not included in Figure 1 and will be given less attention in the section devoted to "tumorigenic mycotoxins." Selection criteria eliminated several mycotoxins that were found in animal experiments as carcinogenic [1, 9–11].

Over 60% of the anticancer agents have been obtained from natural sources [12, 13]. Mycotoxins with antitumor activities have been found in different cell lines [13, 14]. Without striving for completeness, the most important antitumor mycotoxins are:
- mycophenolic acid, penicillic acid, and 5-methoxysterigmatocystin (5-MS) [15],
- anguidine analog scirpenol, di-, and triacetoxyscirpenols [16–18],
- T-2 toxin and related trichothecenes [18],
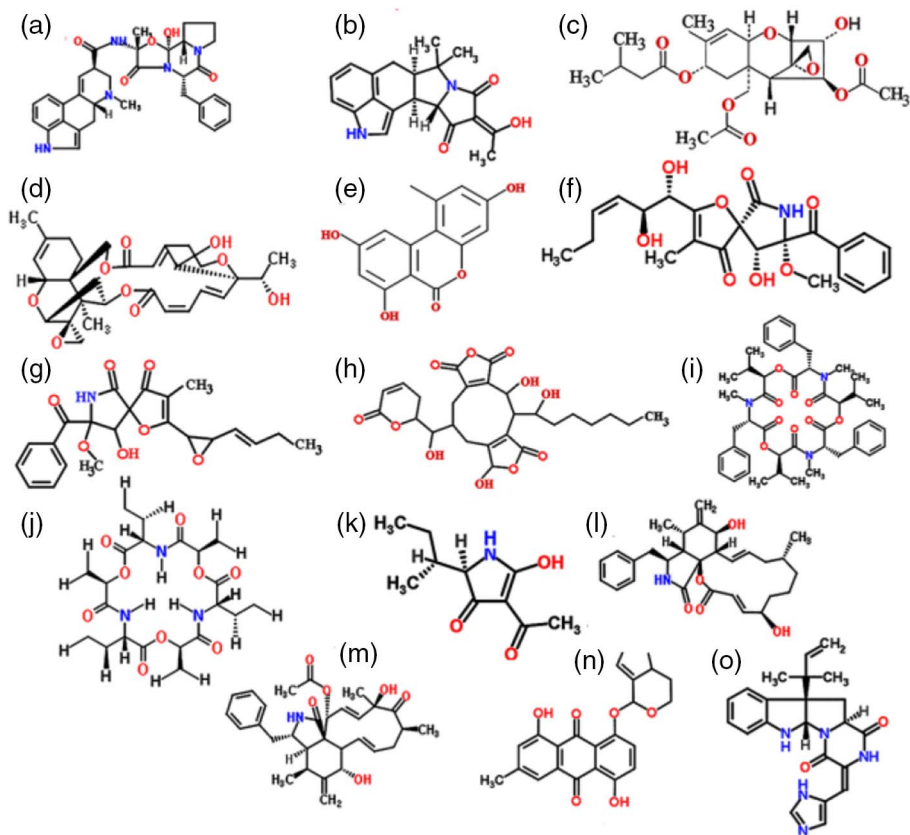
Figure 1. Chemical structures of selected mycotoxins with strong toxic effect and anticancer potential. (a) Ergotamine, (b) cyclopiazonic acid, (c) T-2 toxin, (d) satratoxin H, (e) alternariol, (f) pseurotin, (g) synerazol, (h) rubratoxin, (i) beauvericin, (j) enniatin, (k) tenuazonic acid, (l) cytochalasin B, (m) cytochalasin C, (n) MT81, and (o) roquefortine of beneficial low toxicity

- cytocholasin B [19],
- patulin [20],
- hydromytoxin B and 16-hydroxyroridin E [21],
- tenuazoic acid [22],
- betaacetoxyscirpendiol [23],
- gliotoxin [24],
- fluorinated pseurotin A [25],
- synerazol [26],
- rubratoxin B [27],
- beauvericin [28],

– the macrocyclic trichothecenes verrucarin A and roridin A [29],
– leuteoskyrin, a hydroxyanthraquinone derivative related to MT81 [30].

Adverse effects of mycotoxins

Mycotoxins act like "double-edged swords," and are regarded as "Janus-faced" molecules due to their adverse effects. This definition refers to the harmful genotoxic (mutagenic, teratogenic, and carcinogenic) effect of mycotoxins on the one hand [3, 10] and cytocidal and antineoplastic effects of mycotoxins on the other hand [31–33]. The potential pharmacological application of molecules behaving as "double-edged swords" was tested in at least 300 identified mycotoxins, but only around 30 of them were investigated for their toxicity [34]. This number has been increased since, but only the trivial and IUPAC names of highly toxic and best-known mycotoxins are summarized in Table 1. Many of the mycotoxins display significant *in vitro* inhibition against human cancer cell lines [35]. The clinical aspects of fungal cancer drugs and their synthetic derivatives have also been reviewed [14], despite the tremendous amount of research aimed at the identification of fungal metabolites with potential anticancer activities, the number of fungi-derived agents that have been approved as cancer drugs remained limited [14, 35]. Correspondingly, an even lower number of secondary metabolites have been investigated due to the typical fermentation scale-up challenges in bioreactors [36].

The quantity of metabolites produced by fungi and the cost of their production cause a further constraint against the advancement to clinical stages [14]. As a result of these obstacles, no fungi-derived agent has been approved as an anticancer drug to date in spite of the research in the field aimed at the utilization of fungal metabolites with promising antitumor activities.

The toxic effects of mycotoxins not only question their use but also raise ethical problems that have not been addressed earlier. The question emerged whether or not the antitumorigenic effect of toxic mycotoxins could be utilized against cancer in spite of their DNA damaging, genotoxic "side effects." Regarding ethical considerations related to the medical use of mycotoxins, only limited information is available. In the first BIOMYCO study that raised ethical questions, a multitoxin approach was applied. The mycotoxin exposure in adults and children on a large scale was approved by the ethical committee of the Ghent University Hospital. Within this study, only the design and methods were restricted [37]. The question was whether the combined intake of myco-toxins would lead to a possible higher risk for adverse health effects than the intake of one of these mycotoxins alone [38]. Ethical obstacles against the clinical use of mycotoxins can be overridden only by the permission of ethical committees of hospitals and subsequent recruitment of voluntary participants to

Table 1. Trivial and IUPAC names of selected mycotoxins

Trivial name	IUPAC name
Aflatoxin B_1:	(6aR,9aS)-4-methoxy-2,3,6a,9a-tetrahydrocyclopenta[c]furo[3′,2′:4,5]furo[2,3-h]chromene-1,11-dione
Aflastatin A:	(5R)-5-[(30E,32E)-32-(1,5-dimethyl-2,4-dioxo-3-pyrrolidinylidene)-2,3,4,5,6,8,10,12,14, 16,18,20,22,24,25, 32-hexadecahydroxy-13,15,19,21,23,27,29,31-octamethyl-30-dotriaconten-1-yl]-1-C-(2-hydroxyundecyl)-α-D-lyxopyranose
Aflastatin B:	(5R)-5-[(30E,32E)-32-(5-methyl-2,4-dioxo-3-pyrrolidinylidene)-2,3,4,5,6,8,10,12,14,16,18,20,22,24,25, 32-hexadecahydroxy-13,15,19,21,23,27,29,31-octamethyl-30-dotriaconten-1-yl]-1-C-(2-hydroxyundecyl)-α-D-lyxopyranose
Alternariol:	3,7,9-trihydroxy-1-methyl-6H-benzo[c]chromen-6-one
Anguidine:	(3β,4α,12R)-3-hydroxy-12,13-epoxytrichothec-9-ene-4,15-diyl diacetate
Beauvericin:	(3S,6R,9S,12R,15S,18R)-3,9,15-tribenzyl-6,12,18-triisopropyl-4,10,16-trimethyl-1,7,13-trioxa-4,10,16-triazacyclooctadecane-2,5,8,11,14,17-hexone
Blasticidine S:	4-amino-1-(4-{[(3S)-3-amino-5-(N-methylcarbamimidamido) pentanoyl]amino}-2,3,4-trideoxy-β-D-erythro-hex-2-enopyranuronosyl)-2(1H)-pyrimidinone
Citrinin:	(3R,4S)-8-hydroxy-3,4,5-trimethyl-6-oxo-4,6-dihydro-3H-isochromene-7-carboxylic acid
Cyclopiazonic acid:	(6aR,10Z,11aS,11bR)-10-(1-hydroxyethylidene)-7, 7-dimethyl-6a,7,11a,11b-tetrahydro-6H-pyrrolo[1′,2′:2,3] isoindolo[4,5, 6-cd]indole-9,11(2H,10H)-dione
Cytochalasin Bx:	(3E,5R,9R,11E,12aS,13S,15S,15aS,16S,18aS)-16-benzyl-5,13-dihydroxy-9,15-dimethyl-14-methylene-6,7,8,9,10,12a,13,14, 15,15a,16,17-dodecahydro-2H-oxacyclotetradecino[2,3-d]isoindole-2,18(5H)-dione
Cytochalasin D:	(3S,3aR,4S,6S,7E,10S,12R,13E,15R,15aR)-3-benzyl-6,12-dihydroxy-4,10,12-trimethyl-5-methylene-1,11-dioxo-2,3,3a,4,5,6, 6a,9,10,11,12,15-dodecahydro-1H-cycloundeca[d]isoindol-15-yl acetate
Deoxynivalenol:	(3β,7α)-3,7,15-trihydroxy-12,13-epoxytrichothec-9-en-8-one
Diacetoxyscirpenol:	(3α,4β)-3-hydroxy-12,13-epoxytrichothec-9-ene-4,15-diyl diacetate
Diacetylnivalenol:	3,7-dihydroxy-8-oxo-12,13-epoxytrichothec-9-ene-4,15-diyl diacetate
Ergotamine:	(5′α)-5′-benzyl-12′-hydroxy-2′-methyl-3′,6′,18-trioxoergotaman

Table 1. Trivial and IUPAC names of selected mycotoxins *(Continued)*

Trivial name	IUPAC name
Fumonisine B$_1$:	(2R,2′R)-2,2′-{[(5R,6R,7S,9S,11R,16R,18S,19S)-19-ammonio-11,16,18-trihydroxy-5,9-dimethyl-6,7-icosanediyl]bis[oxy(2-oxo-2,1-ethanediyl)]} disuccinate
Fusarenon:	(3β,4α,7α)-3,7,15-trihydroxy-8-oxo-12,13-epoxytrichothec-9-en-4-yl acetate
Gliotoxin:	(1R,7S,8S,11R)-7-hydroxy-11-(hydroxymethyl)-15-methyl-12,13-dithia-9,15-diazatetracyclo[9.2.2.0^{1,9}.0^{3,8}]pentadeca-3,5-diene-10,14-dione
HT-2 toxin:	(3α,4β,8α,12R)-15-acetoxy-3,4-dihydroxy-12,13-epoxytrichothec-9-en-8-yl 3-methylbutanoate
5-methoxysterigmatocystin (5-MS):	(3aR,12cS)-8-hydroxy-6,11-dimethoxy-3a,12c-dihydro-7H-furo[3′,2′:4,5]furo[2,3-c]xanthen-7-one
MT81:	1,5-dihydroxy-3-methyl-8-(2,6,7,7a-tetrahydro-4H-furo[3,2-c]pyran-4-yloxy)-9,10-anthraquinone
Mycophenolic acid:	(4E)-6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydro-2-benzofuran-5-yl)-4-methyl-4-hexenoic acid
Neosolaniol:	(3α,4β,8α,12R)-3,8-dihydroxy-12,13-epoxytrichothec-9-ene-4,15-diyl diacetate
Nivalenol:	[(3β,4α,7α,12ξ)-3,4,7,15-tetrahydroxy-12,13-epoxytrichothec-9-en-8-one
O-acetyl-5-MS:	(3aR,12cS)-8-ethylacetate-6,11-dimethoxy-3a,12c-dihydro-7H-furo[3′,2′:4,5]furo[2,3-c]xanthen-7-one
Ochratoxin A:	N-{[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydro-1H-isochromen-7-yl]carbonyl}-L-phenylalanine
Patulin:	(hemiacetal form) (4-hydroxy-4H-furo[3,2-c]pyran-2(6H)-one
Penicillic acid:	(2E)-3-methoxy-5-methyl-4-oxo-2,5-hexadienoic acid
Pseurotin A:	(5S,8S,9R)-8-benzoyl-2-[(1S,2S,3Z)-1,2-dihydroxy-3-hexen-1-yl]-9-hydroxy-8-methoxy-3-methyl-1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione
Roquefortine C:	(3E,5aS,10bR,11aS)-3-(1H-imidazol-4-ylmethylene)-10b-(2-methyl-3-buten-2-yl)-6,10b,11,11a-tetrahydro-2H-pyrazino[1′,2′:1,5]pyrrolo[2,3-b]indole-1,4(3H,5aH)-dione
Rubratoxin A:	4,8-dihydroxy-5-(1-hydroxyheptyl)-10-[hydroxy(6-oxo-3,6-dihydro-2H-pyran-2-yl)methyl]-4,5,8,9,10,11-hexahydro-1H-furo[3′,4′:5,6]cyclonona[1,2-c]furan-1,3,6-trione
Satratoxin H:	(2S,6′R,11′R,13′R,15′S,16′R,19Z,21′E,23′R,27′R)-Hydroxy-23′-[(1S)-1-hydroxyethyl]-9′,15′-dimethyl-3′H,18′H-spiro[oxirane-2,14′-[4,12,17,24]tetraoxapentacyclo [21.3.1.1^{13,16}.0^{6,11}.0^{6,15}]octacosa[1,9,19,21]tetraene]-3′,18′-dione

Sterigmatocystin: (3aR,12cS)-8-hydroxy-6-methoxy-3a,12c-dihydro-7H-furo[3′,2′:4,5]furo [2,3-c]xanthen-7-one

Synerazol: 8-benzoyl-2-{3-[(1E)-1-buten-1-yl]-2-oxiranyl}-9-hydroxy-8-methoxy-3-methyl-1-oxa-7-azaspiro[4.4]non-2-ene-4,6-dione

T-2 toxin: 4,15-diacetoxy-3-hydroxy-12,13-epoxytrichothec-9-en-8-yl 3-methylbutanoate

Tenuazonic acid: 3-acetyl-5-sec-butyl-4-hydroxy-1,5-dihydro-2H-pyrrol-2-one

Xanthone: 9H-xanthen-9-one

Zearalenone: (3S,11E)-14,16-dihydroxy-3-methyl-3,4,5,6,9,10-hexahydro-1H-2-benzoxa-cyclotetradecine-1,7(8H)-dione

test antitumor mycotoxins being at least in Clinical Trial Phase II upon the approval of patient(s). To shortcut the viewpoint of patients with incurable cancers, it is predicted that patients do not care too much about future consequences of toxic side effects of a drug when it provides an immediate relief of pain and/or significantly extends the lifespan. Reliable anticancer drugs have been found more than 50 years ago to prolong the lifespan of animals [39] and by analogy, this can be expected for their human use, but in this regard, not much progress has been made. Without further discussion of mycotoxins as antitumor agents, the opinion of terminally ill patients is acceptable only for elder patients, who would not represent a serious risk to the reproductive cycle by spreading mycotoxin-affected mutations to their children. Considering this attitude of metastatic patients, the review primarily focuses on neoplastic, cytotoxic, anticancer aspects of mycotoxins, and other tolerable side effects.

Tumorigenic Mycotoxins

The common and IUPAC systematic names of mycotoxins are summarized in Table 1. As systematic names can be convoluted and are difficult to remember, the use of their trivial names is preferred. The exclusion of mycotoxins that have genotoxic (mutagenic, teratogenic, and carcinogenic) and immunosuppressive properties will affect several compounds in conformity with earlier animal experiments suggesting that most of the mycotoxins are carcinogens [1, 8, 9, 40]. Table 1 contains the most important tumorigenic mycotoxins, such as aflatoxins, patulin, penicillic acid (not to be confused with penicillin G or V), gliotoxin, citrinin, fumonisin, and ochratoxin. However, these frequently occurring mycotoxins also exert antitumor activities in different cell lines and in *in vivo* experiments, including aflatoxin, patulin, penicillic acid, gliotoxin, roquefortine C, trichothecenes, trichothecene analogs of anguidine, cytochalasins, 14-hydromytoxin B and 16-hydroxyroridin E, tenuazonic acid, pseurotin A, synerazol, rubratoxin B, beauvericin, 5-methoxysterigmatocystin, and mycophenolic acid. Corresponding to this "double sword effect," often the same compounds can be found in the listing of both the tumorigenic and antitumorigenic groups. In such cases, the tumorigenic effect should be a slow process and by no means an acute one, whereas the antitumorigenic cytotoxic effect is expected to be instant without serious side effects.

Mycotoxins with serious tumorigenic effects (aflatoxin, patulin, penicillic acid, and gliotoxin) are thus automatically excluded from the potential list of antimetastatic agents (Table 2).

Table 2. Structural and toxicological relationship of mycotoxins

Selected compouds	Ring structure	Number of heteroatoms (in rings)	Number of oxygen atoms (total)	Keto (=CO)	Hydroxyl (-OH)	Carboxyl (-COOH)	Amino (-NH2)	Imino (=NH)	Genotoxicity 1a. mutagenic 1b. teratogenic 1c. carcinogenic	Cellular toxicity 2a. high 2b. low 2c. apoptotic and necrotic	Antitumorigenic potential 3
Aflatoxin B1	Polyketide, 5-membered ring	3 O	6	2	0	0	0	0	1c		
Citrinin	Isochromene, polyketide	1 O	5	1	1	1	0	0	1a, 1c	2a	
Fumonisine B1	Disuccinic acid derivative	None	15	2	3	4	1	0	1a		
Ochratoxin A	Pentaketide dihydrocoumarin	1 O	6	2	0	1	0	1	1a, 1b	2b	
HT-2 toxin	Type A trichothecene	2 O	7	0	2	1	0	0	1a		
Satratoxin	Type A trichothecene	4 O	9	2	2	0	0	0	1a		
T-2 toxin	Type A trichothecene	2 O	9	2	1	2	0	0		2a	
Synerazol	Heterospirocyclic γ-lactam	2 O, 1 N	7	3	1	0	0	0		2a	
Neosolaniol	Type A trichothecene	**2 O**	**8**	**0**	**2**	**1**	**0**	**0**		**2a**	
Nivalenol	Type B trichothecene	**2 O**	**7**	**1**	**4**	**0**	**0**	**0**		**2a**	
Fusarenon-X	Type B trichothecene	**2 O**	**9**	**1**	**3**	**1**	**0**	**0**		**2a**	
Penicillic acid	Isoprenyl-methoxy-furan	1 O	4	1	1	1	0	0		2a	
Roquefortin C	Cyclodipeptide	5 N	2	2	0	0	0	0		2b	
Diacetylnivalenol	Type B trichothecene	2 O	9	1	2	2	0	0		2b	
Deoxynivalenol	Type B trichothecene	1 O	5	1	2	0	0	0		2b	

(Continued)

Table 2. Structural and toxicological relationship of mycotoxins *(Continued)*

Selected compounds	Ring structure	Number of hetero-atoms (in rings)	Number of oxygen atoms (total)	Number of functional groups					Genotoxicity 1a. mutagenic 1b. teratogenic 1c. carcinogenic	Cellular toxicity 2a. high 2b. low 2c. apoptotic and necrotic	Antitumorigenic potential
				Keto (=CO)	Hydroxyl (-OH)	Carboxyl (-COOH)	Amino (-NH2)	Imino (=NH)			
Zearalenol	Type B trichothecene	1 O	5	2	2	0	0	0		2b	3
Beavericin	Cyclic hexadepsipeptides	1 N	9	6	0	0	0	0		2b	
Cytochalasin B	Macrocyclic ring	1 N, 1 O	5	2	2	0	0	0		2b	3
Gliotoxin	Diketopiperazine derivatives	2 N, 2 S	4	2	1	0	0	0		2c	3
Ergotamine	Tetracyclic ergoline	4 N, 1 O	5	3	1	0	0	1			3
Cyclopiazonic acid	Pentacyclic indole tetramic acid	2 N	3	2	1	0	0	0			3
Diacetoxyscirpenol	Trichothecenes (anguidine)	**2 O**	**7**	**0**	**1**	**2**	**0**	**0**			**3**
Pseurotin A	Heterospirocyclic γ-lactam	2 O, 1 N	8	3	3	0	0	0			3
Rubratoxin A	Pyrano-cyclononal-furan	3 O	11	4	4	0	0	0			3
5-MS Sterigmatocystin	Xantone derivative	3 O	7	1	1	0	0	0			3
O-Acetyl-5-MS	Xantone derivative	3 O	8	1	0	1	0	0			3
Sterigmatocystin	Xantone derivative	3 O	6	1	0	0	0	0			3
Mycophenolic acid	Dihydrobenzofuran	1 O	8	1	0	1	0	0			3
Tenuazoic acid	Dihydro-pyrrol	1 N	3	1	1	0	0	0			3
Cytochalasin D	Macrocyclic ring	1 N	6	2	2	0	0	0			3
Patulin	Hydroxy-furopyranone	2 O	4	1	1	0	0	0			3
MT81	Tetrahydro-pyran-anthraquinone	2 O	7	2	2	0	0	0			3

Note: Bold values indicate potential antineoplastic trichothecenes with high cyctotoxicity.

The nephrotoxic, hepatotoxic, and cancer-promoting effects expel citrinin from using it as an antineoplastic agent. Similarly, the strong carcinogenic, teratogenic, nephrotoxic character of ochratoxin, and other foodborne mycotoxins disqualify themselves from being considered as cytotoxic agents against tumor growth (Table 2). Fumonisin is excluded because of the probable link between fumonisin toxicity and esophageal cancer [41].

Although satratoxins are extremely toxic, satratoxin H (Figure 1d) will be given more attention among type D trichothecenes.

On the contrary, it would be a futile effort to turn mild or beneficially toxic mycotoxins into antitumor agents (e.g., roquefortin C). Tumorigenic mycotoxins are not discussed in detail, but their detrimental effects deserve mention. A viable strategy could be the reduction or minimization of the extreme toxicity of antitumorigenic mycotoxins by:

(1) modification of mycotoxins through chemical, physical, and biological methods;
(2) inhibiting mutagenesis and carcinogenesis by applying antimutagenic agents and to prevent the interaction of mycotoxins with DNA [42];
(3) agents, which bind mycotoxins and prevent absorption by the animals' digestive tract [43–49]. Agents binding to mycotoxins are expected to be approved by the FDA for the prevention or treatment of mycotoxicoses.

Antitumor Mycotoxins

Ergot alkaloids

Ergot alkaloids are produced by *Claviceps*, *Epichloë, Penicillium*, and *Aspergillus* spp. [50] and cover a wide range of biological activities with effects on blood circulation and neurotransmission to mention just the most important ones [51]. Chemically, the ergot alkaloids are characterized by the presence of a tetracyclic ergoline ring (Figure 1a). Genotoxicity studies have been carried out only in ergotamine (Figure 1a) but not in other naturally occurring ergot alkaloids. Although ergotamine did not initiate bacterial or mammalian cell mutation, earlier studies showed some weak and inconsistent chromosome damaging effects *in vitro* and *in vivo*. Mutagenicity testing of ergot alkaloids has shown that these compounds have no mutagenic potential [52–55]. In spite of these claims, chromosomal damages were reported by Roberts and Rand [56, 57], whereas others concluded that the genotoxicity and carcinogenicity of ergot alkaloids showed a non-genotoxic mode of action [58]. Considering the cytotoxicity of clavines as the preferred mycotoxic effect, they could be potential antitumor agents [59].

Cyclopiazonic acid

Another fungal secondary metabolite, structurally related to clavines, is the indole alkaloid cyclopiazonic acid (Figure 1b) [60]. The antimalarial drug cyclopiazonic acid was first isolated from *Penicillium* species (*P. cyclopium*, *P. griseofulvum*, *P. camemberti*, and *P. commune*) and subsequently from *Aspergillus flavus*, *Aspergillus versicolor*. This cell permeable ergot-like alkaloid is a reversible inhibitor of the sarcoendoplasmic (SERCA) reticulum and sarcoplasmic Ca^{2+} ATPases [61] and could be utilized in combination with other agents against tumor and metastatic developments.

Trichothecenes

Trichothecenes are generally formed *via* the mevalonate pathway. These are sesquiterpene analogs and constitute one of the major groups of mycotoxins, if not the largest one. Correspondingly, these compounds will be given more attention. The macrocyclic and non-macrocyclic trichothecenes constitute a family of more than 60 metabolites produced by species from a number of fungal genera, including Fusarium, Myrothecium, Phomopsis, Stachybotrys, Trichoderma, Trichothecium, and others [62–64]. The basic structure of sesquiterpenoid trichothecenes has been subdivided into types A, B, C, and D groups [65].

The chemical structures of major derivatives of types A and B trichothecenes are shown in Figure 2. The type A group includes diacetoxyscirpenol, T-2 toxin, and neosolaniol. The type B group contains a ketone at the C-8 position and includes nivalenol, fusarenon-X, and diacetylnivalenol. Diacetonivalenol is the natural acetylated derivative of deoxynivalenol [66].

T-2 Mycotoxin. The best-known representative of type A trichothecenes is T-2 mycotoxin (Fusariotoxin T2) [67] (Figure 1c) a widespread contaminant of grain and grain products. T-2 toxin is produced by species of *Fusarium*, *Myrothecium*, *Trichoderma*, *Trichothecium*, *Cephalosporium*, *Verticimonosporium*, and *Stachybotrys*. Besides deoxynivalenol, T-2 is the most toxic trichothecene. These two toxins cause the most concern due to their wide distribution. T-2 toxin is a potent mycotoxin produced primarily by the species of the genus *Fusarium* and elicits inflammatory and genotoxic effects. The toxicity of T-2 toxin is related to its 12,13-epoxy ring [67] that could be connected to its oxidative stress-mediated cytotoxicity [11]. The structure–activity relationship revealed that the free hydroxy in the C-3 position present in type A and type B trichothecenes contributed to cytotoxicity. Free hydroxy groups at the 4th, 8th, and 15th positions probably interfered with the cytotoxicity, as their esterification resulted in improved cytotoxicity [68]. Cell cycle analysis proved the apoptotic effect of T-2 toxin
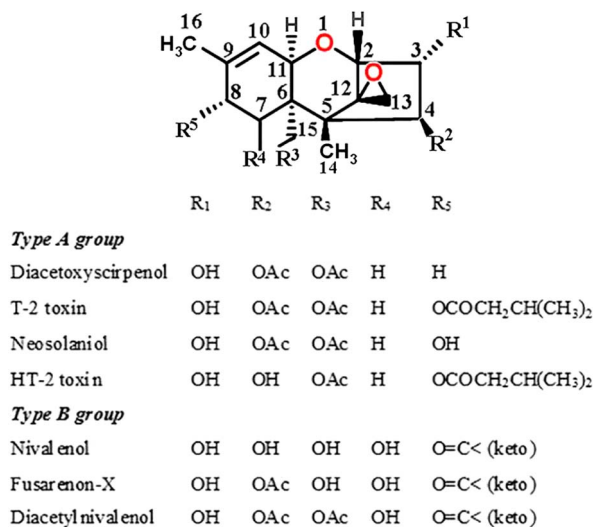
	R₁	R₂	R₃	R₄	R₅
Type A group					
Diacetoxyscirpenol	OH	OAc	OAc	H	H
T-2 toxin	OH	OAc	OAc	H	OCOCH$_2$CH(CH$_3$)$_2$
Neosolaniol	OH	OAc	OAc	H	OH
HT-2 toxin	OH	OH	OAc	H	OCOCH$_2$CH(CH$_3$)$_2$
Type B group					
Nivalenol	OH	OH	OH	OH	O=C< (keto)
Fusarenon-X	OH	OAc	OH	OH	O=C< (keto)
Diacetylnivalenol	OH	OAc	OAc	OH	O=C< (keto)

Figure 2. Chemical structures of trichothecene analogs

and its metabolites (HT-2 and adenosolaniol) in micromolar concentrations [69]. The metabolic network of trichothecene mycotoxins is quite diverse. Related studies would require significant efforts to understand their fate and modify their toxicity in animals and humans [70].

T-2 mycotoxin was suspected to have been used as a chemical warfare agent from the 1970s till the 1990s and delivered by low-flying aircraft releasing a yellow oily liquid causing a phenomenon named "yellow rain." Others proposed that the "yellow rain" found in Southeast Asia was originated from the excrement of jungle bees [71]. T-2 mycotoxins that can be absorbed through the skin have been officially classified and stigmatized as chemical weapons. Nevertheless, the trichothecene cytotoxicity of type A (T-2 and HT-2), type B (deoxynivalenol and nivalenol), and type D (satratoxin G and H) toward different cell lines was within a narrow limit between 4 nM and 5 μM IC50 concentration range, suggesting that the potential of trichothecenes as antitumor agents has been underestimated [72].

Satratoxin H. This trichothecene mycotoxin (Figure 1d) is a naturally occurring byproduct of the mold *Stachybotrys chartarum* that is toxic to both humans and animals causing a clinical condition known as *Stachybotrotoxicosis*. The six-membered rings of satratoxin H are related to mycotoxin T-2, but unlike T-2, it has not been reported to have been used as a biological weapon. Although the apoptotic effects of satratoxin H mediated through double-stranded DNA breaks could be exploited, the induction of genotoxicity suggests a considerable

genotoxic risk [73]. Satratoxin H is about five times more toxic than the T-2 toxin. The extreme toxicity of satratoxin H was explained by the generation of reactive oxygen species (ROS) and lipid peroxides leading to apoptosis in PC12 cells [74].

Anguidine analogs. The mycotoxin anguidine (4,15-diacetoxyscirpenol,4, 15-DAS) is a highly oxygenated trichothecene that was first isolated from *Fusarium equiseti* [75]. This trichothecene has been transformed to several closely related derivatives through the combination of microbial and chemical modifications [16, 17] (Figure 3). Anguidines have been subjected to microbial transformations by *Mucor mucedo*, *Streptomyces griseus*, *Acinetobacter calcoaceticus*, and *Fusarium oxysporum* strains. The antitumor activity of anguidine observed in leukemic mice [76] was tested in human phase I clinical trial, but anguidine caused an unexpected pain syndrome [77, 78]. Moreover, the antitumor activity of the trichothecenes was minimal or absent in patients treated with anguidine [17]. Because of their painful toxicity, the life-threatening hypotensive effects, and the poor tolerance by patients, the use of trichothecenes as chemotherapeutic drugs was discontinued.

Due to the disappointing results in clinical trials, the lethal and cytokinetic effects of anguidine were tested on cultured human colon cancer cells, but its tumoricidal activity was poor even after prolonged exposure [79]. The National Cancer Institute has determined that the trichothecene mycotoxin anguidine is a potent teratogen (http://www.cancer.gov/publications/dictionaries/cancer-drug?cdrid=39148). This did not completely eliminate anguidine as a potential antitumor–antimetastatic agent from the race, as teratogenicity causes congenital

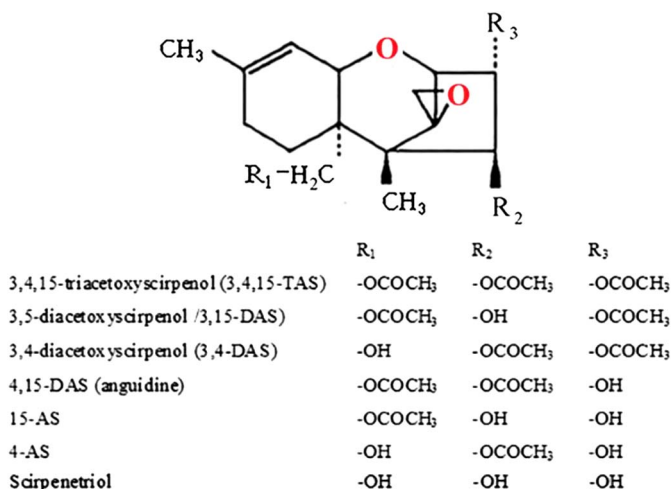|	R_1	R_2	R_3
3,4,15-triacetoxyscirpenol (3,4,15-TAS)	-OCOCH₃	-OCOCH₃	-OCOCH₃
3,5-diacetoxyscirpenol /3,15-DAS)	-OCOCH₃	-OH	-OCOCH₃
3,4-diacetoxyscirpenol (3,4-DAS)	-OH	-OCOCH₃	-OCOCH₃
4,15-DAS (anguidine)	-OCOCH₃	-OCOCH₃	-OH
15-AS	-OCOCH₃	-OH	-OH
4-AS	-OH	-OCOCH₃	-OH
Scirpenetriol	-OH	-OH	-OH

Figure 3. Anguidine analogs of trichothecenes

malformation, whereas metastatic transformations of tumors more frequently occur in elder patients. To find the active chemical group(s) responsible for teratogenicity, approximately 60 derivatives of anguidine were tested to select them for antitumor activities. Structural positions 3, 4, 8–10, and 15 were modified, and the resultant derivatives were screened against leukemia cells. It was found that the introduction of C3-keto, C3, and C8-diketo groups markedly improved the antileukemic activity, whereas epoxidation of the C9–C10 double bond or oxidation of the C15 position diminished the antileukemic activity [18].

Anguidines are "Janus-faced" compounds representing a group of trichothecene mycotoxins exerting both antitumor and teratogenic effects. Nevertheless, the concentration- and time-dependent universal cell cycle arrest of anguidine could be improved in combination with other antimetabolites to reduce the toxicity of a variety of subsequently administered agents, including ara-C, adriamycin, 5-fluorouracil, and hydroxyurea [80, 81].

Relations of chemical structures of other trichothecene toxins to their biological activities were also investigated. In verrucarol (scirpendiol), scirpentriol and other hydroxylated verrucarin analogs, the esters significantly showed higher cytotoxicity. Apoptosis was induced by 4-beta-acetoscirpendiol produced by *Paecilomyces tenuipes* in human leukemia cell lines *in vitro* [23].

Minor trichothecenes. Type C minor trichothecenes have a C-7/C-8 epoxide (e.g., crotocin). Similar to satratoxin H, type D minor trichothecenes have an additional ring bridging the C-4 and C-15 positions (e.g., roridin A and verrucarin A) [63]. Verrucarin, a type D trichothecene, is highly toxic due to the basic trichothecene structure and the presence of olefin, epoxide, keto groups, and R substituted hydroxy groups. Types C and D trichothecenes are less well known (structures not shown).

Among additional trichothecenes 14′-hydroxymytoxin B and 16-hydroxyroridin E were isolated from *Myrothecium roridum* and showed potent cytotoxic activity against soft-tissue sarcoma cells [21]. The controversy surrounding the use of trichothecenes [82] is illustrated by the various effects of trichothecenes causing digestive symptoms (diarrhea and emesis), weight loss, nervous disorders, bone marrow damage, cardiovascular alterations, hemostatic changes, and immune suppression [83, 84]. More importantly, liver damages, hepatotoxicity, carcinogenic, and teratogenic effects were not among these disorders. The analogs of trichothecenes that have been synthesized [62] carry the promise that the fine-tuning of modifications could result in antitumor candidates. Elimination by detoxification could be achieved by extensive and rapid biotransformation in the kidney/liver (3:1), but other cells also actively metabolize these trichothecene toxins normally by deacylation, deepoxidylation, and oxidation at the C-12 and C-13 position in the basic structure [85–87]. By the removal of oxygen from the

epoxide groups, trichothecene mycotoxin deepoxy metabolites could be formed that are essentially non-toxic [86].

Although the use of warfare trichothecenes has drastically been reduced, subacute doses of trichothecene mycotoxins could still serve biological warfare. Chronic toxicity has iatrogenically been induced by repeated subacute doses of trichothecene mycotoxins administered intravenously to cancer patients as a chemotherapy for colon adenocarcinoma. No conclusive reports were published. Nevertheless, low doses of non-toxic trichothecenes in combination with other synergistic mycotoxins could be beneficial against metastatic tumor spread, particularly by the selection of right doses and proper administration. As far as trichothecenes are concerned, by ignoring anecdotal evidence, one can conclude that these mycotoxin analogs remain potential candidates as antineoplastic agents after matching the functional demand with the structural design.

Deoxynivalenol and zearalenone. Mycotoxins of Fusarium graminearium produce among other mycotoxins deoxynivalenol and zearalenone. Deoxynivalenol is one of the most prevalent cereal contaminants with major public health concerns [88]. Poultry and swine are among the most sensitive species for deoxynivalenol and zearalenone mycotoxins [88–90] (http://www.trilogylab.com/uploads/ Mycotoxin_CAST_Report.pdf). Deoxynivalenol, a type B trichothecene derivative interferes with cell growth, alters brain neurotransmitter production, immunity, and causes organ damage. On the other hand, zearalenone, a phenolic resorcylic acid lactone, resembling the structure of estradiol-17β, results in embryonic death, smaller litters, and smaller offspring of pigs [89, 91, 92].

The treatment of pigs with the oxidative stress inductor deoxynivalenol and zearalenone mycotoxins increased the level of the 8-OHdG carcinogenic risk factor, whereas yeast-based feed additives tended to compensate the effect of 8-OHdG upon mycotoxin treatment [93]. Deoxynivalenol is known to cause oxidative stress [11, 94] and a spectrum of diseases in animals and humans by affecting the signaling pathways in cells [95]. Emerging evidence suggested that deoxynivalenol produced its toxicity primarily *via* activation of the mitogen-activated protein kinase signaling pathway and altering the expression of genes responsible for key physiological and immunological functions of the intestinal tissues of chickens and pigs. These findings highlighted the cytotoxic risks associated with the intake of even low levels of deoxynivalenol and also indicated the gap of knowledge that needs to be addressed in future research [88].

Zearalenone is undoubtedly implicated in reproductive disorders of swine and other domestic animals. Experiments *in vivo* and *in vitro* indicate that type B zearalenone and its metabolites exert estrogenic effects resulting in

functional and morphological alterations in reproductive organs [96]. Consequently, those trichothecenes (deoxynivalenol, T-2, and zearalenone) and fumonisin toxins that affect ovarian and testicular function, placenta and fetus, puberty and sexual maturity of domestic animals are unlikely to be used as antimetastatic agents.

Alternariol

The toxic secondary fungal metabolite alternariol (Figure 1e) is produced by *Alternaria* molds. It is cytotoxic, fetotoxic, teratogenic, mutagenic, and genotoxic [97]. Alternariol was reported to induce apoptosis in mouse hepatoma cells [98]. Insufficient knowledge regarding the cellular effects of alternariol requires caution before its anticancer and antimetastasis potential can be realistically judged and taken into consideration.

Spirocyclic γ-lactams

Pseurotin A. The expression of pseurotin A (Figure 1f) as a secondary metabolite of *Aspergillus* and other fungi [99] is induced in response to hypoxia [100]. The spirocyclic γ-lactam pseurotin A showed a moderate effect against phytopathogenic bacteria and presented low cytotoxicity toward human lung fibroblasts [101]. Pseurotin and its analog synerazol are inhibitors of immunoglobulin E [102].

Synerazol. This mycotoxin (Figure 1g) is active against *Candida albicans* and other fungi synergistically activated with azole-type antifungal agents [26]. Its activity was potentiated by the co-presence of azole compounds, such as the antifungal clotrimazole (chlortrityl imidazole) applied topically [103].

Rubratoxin A (Figure 1h) is a pyrano-cycloclonal-furan derivative and a potent, selective, competitive inhibitor of protein phosphatase 2A, inducing a caspase-dependent decrease in cell viability and *in vivo* antitumor effect [104–106]. As a cytostatin analog, rubratoxin A could be a basic compound for chemical modifications leading to the development of antitumor–antimetastasis drugs based on the strategy that is targeting protein phosphatase 2A [105].

Beauvericin (Figure 1i) is a cyclic peptide, toxic to human cell lines [107, 108], induces apoptosis and DNA fragmentation [108, 109]. Beauvericin causes cytotoxic effects in human acute lymphoblastic leukemia cells [28]. Unfortunately, it also exerts a strong negative inotropic effect, i.e., decreases the cardiac contraction strength [110]. Beauvericin inhibited the migration of the metastatic prostate cancer (PC-3M) and breast cancer (MDA-MB-231) cells and showed antiangiogenic activity in HUVEC-2 cells at sublethal concentrations

[111]. Beauvericin also inhibited the growth of KB and KBv200 cells, induced apoptosis through the intrinsic mitochondrial pathway by the generation of the ROS, loss of mitochondrial membrane potential, the release of cytochrome c, activation of caspase-9 and -3, and cleavage of PARP [112]. The channel forming ability of beauvericin and enniatin selectively directs a flux of cations – particularly calcium – into the cell. The increased intracellular calcium levels might be at least in part responsible for their cytotoxicity [33]. The cytotoxic effects of beauvericin leading to apoptosis could be utilized against tumor growth, but to avoid its strong negative inotropic effect, a lower dose in combination with other anticancer drugs is recommended.

Enniatin. The circular arrangement of the cyclic depsipeptide enniatin A (Figure 1j) structure and other major enniatins (Enn A1, B, B1), as well as minor enniatins resemble beauvericin. As expectable from the circular form and empty hole inside their structures, enniatins act as ionophores [113]. Profound p53-dependent cytostatic and p53-independent cytotoxic activities, especially against human cancer cells, suggested a potential of enniatin as an anticancer drug [31]. It was confirmed that enniatins A1 and B1, and to a lesser extent, enniatin B may possess anticarcinogenic properties by the induction of apoptosis and disruption of extracellular signal-regulated kinase signaling pathway [114]. The combination of Enn B with the clinically approved multikinase inhibitor sorafenib displayed profound synergistic *in vitro* and *in vivo* anticancer effects against cervical cancer. This finding drew attention to the novel combination strategy particularly for the treatment of cervical cancer [115].

Tenuazonic acid. The mycotoxin tenuazonic acid (Figure 1k) is a dihydropyrrole derivative, produced by *Alternaria* species [116]. It was found to be a powerful eukaryotic protein synthesis inhibitor [117] that significantly delayed the onset of tumorigenesis and also reduced the cumulative number of tumors per tumor-bearing animals. The antitumor and protective potential of tenuazonic acid was utilized against polycyclic aromatic hydrocarbon-induced skin carcinogenesis in mice [22]. The diketo and enol groups bound to the pyrroline ring seem to be crucial for its antitumor activity.

Cytochalasins. These are macrocyclic tetramic acid derivatives. These mycogenic toxins bind filamentous F-actin, thereby block the polymerization and microfilament formation, which in turn affects cellular morphology, inhibits cellular processes such as cell division, and induces apoptosis [118, 119]. Similar to the antibiotic effect of penicillic acid, it was observed that the toxic mold metabolite cytochalasin isolated from *Helminthosporium* species generated binucleate cells, at higher doses caused nuclear extrusion and even total enucleation due to the inhibition of cell division [120]. The *Helminthosporium* genus of fungi belongs to the order of *Pleosporales* (phylum *Ascomycota*) of the kingdom Fungi [121].

The best studied among cytochalasin alkaloids is the cell permeable cytochalasin B (Figure 1l), isolated from the fungus *Helminthosporium dematioideum*. The phytotoxic effect of cytochalasin B deserves mention because of its additive effect with another phytotoxic compound known as pyrenophoric acid that is structurally closely related to abscisic acid, demonstrating the combined action of multiple toxic compounds [122]. From a pharmacological perspective, cytochalasin B strongly inhibits the synthesis of actin filaments, blocking the formation of microfilaments and cytoplasmic division of cells. The inhibition of cell movement and inherent nuclear extrusion has been exploited for cloning through nuclear transfer. Cytochalasin D was more effective than cytochalasin B as a cytoskeletal inhibitor for the production of somatic cell nuclear transfer of embryos in miniature pigs [123]. In the nuclear transfer, the oocytes are aspirated into a micro-needle without rupturing the plasma membrane. However, there is no information regarding the damage to the nucleus upon cytochalasin B treatment, since another cytochalasin, namely cytochalasin C (Figure 1m) is known to cause nuclear damages and binucleate cell formation. More importantly, cytochalasin B was used *in vivo* against murine lymphoma and sarcoma as a single agent or in combination with other chemotherapeutical drugs to amplify the antitumor activity [19, 119]. Due to its unique chemotherapeutic mechanisms, cytochalasin B could be exploited as a promising antineoplastic agent. Based on preclinical murine models [124], cytochalasin B possesses substantial synergistic potential with microtubule- and nucleic acid-directed agents [125]. Murine models showed that the antineoplastic activities of cytochalasin B and D can substantially potentiate chemotherapeutic agents, improve the efficacy of antitumor therapy, and increase the life expectancy of mice [126].

The major effects of cytochalasin D include the inhibition of formation of contractile microfilaments of actin, prevent the separation of the dividing nucleus, and result in multinucleated, primarily binucleated cell formation, inhibition of cellular movement, and induction of nuclear extrusion and enucleation. Additional effects of cytochalasin D are the inhibition of DNA synthesis, increased motility of sperm, glucose transport, and the release of thyroid and growth hormones. Cytochalasin D was reported to inhibit CT26 tumor growth and angiogenesis [127] but promoted pulmonary metastasis of B16 melanoma cells [128]. This controversial observation may indicate that the anticancer and antitumor properties of the same compound may not only depend on its chemical structure but also on its acceptor acting on opposite molecular mechanism of cells.

Recent investigations revealed that new cytotoxic cytochalasans named periconiasins after the fungus *Periconia* sp. F-31 showed cytotoxicity against human HCT-8 cancer cells [129]. Trichodermone, the first spiro-cytochalasan

isolated from the endophytic fungus *Trichoderma gamsii*, displayed moderate (IC50 5.7 μM) inhibitory activity against HeLa cells [130]. Cytochalasins from the medicinal macrofungus *Cordyceps taii* exerted potent antitumor activities against the lung cancer cell 95-D [131]. Other new aspochalasins A–D and aspochalasin Z were regarded as cytochalasan antibiotics. The antibacterial activity of these cytochalasins was weak and showed strong cytostatic effects toward various tumor cells [132], which allowed to categorize them as mycotoxins. Investigations related to the cytotoxic activities of cytochalasins isolated from terrestrial and marine fungi indicated that scientists regarded them the most prospective antitumor agents. Computer-assisted phase-contrast microscopy revealed two groups of cytochalasins, cytotoxic *versus* cytostatic ones. These data opened new *vistas* for tuning cytochalasin targets and to develop non-toxic, cytostatic cytochalasins to combat cancers and metastases associated with poor prognoses [119].

Cytochalasin C (Figure 1m) is another example how chemical changes characterized by the fusion of a macrocyclic ring to a highly substituted isoindole ring drastically reduce the toxicity of other cytochalasins without losing effectiveness against cell growth and potentially against metastasis.

MT81. The furo-pyran-anthraquinone derivative mycotoxin MT81 (Figure 1n) was isolated, purified, and identified from a fungal strain of *Penicillium nigricans* [30]. As expected from the highly toxic mycotoxin, it exerted a bone marrow depressive effect [133] and caused liver, brain, and kidney dysfunctions [134].

More importantly, MT81 has shown *in vitro* and *in vivo* antitumor activities against Ehrlich ascites tumor cells [135]. The Ehrlich ascites carcinoma is a spontaneous murine mammary adenocarcinoma [136] that was treated in inbred mice by serial intraperitoneal (i.p.) administration. In Ehrlich ascites cell-bearing mice, the increased volume of ascites fluid in the peritoneum is the nutritional basis and a necessary factor for tumor cell growth. MT81 and its acetylated analog (Aa-MT81) turned out to decrease the ascites fluid volume and viable cell count and increased the percentage of the lifespan of Ehrlich ascites cell-bearing mice. Aa-MT81 was more potent and less toxic than its parent MT81 compound. Abdominal tumors generate often ascites, thus the antineoplastic and antioxidant activities of mycotoxins were tested against Ehrlich ascites carcinoma in mice. As expected MT81 and its structural hydroxyanthraquinone analog, luteoskyrin proved to be potent inhibitor against Ehrlich ascites tumor cells [30, 137]. Luteoskyrin is a hepatotoxic mycotoxin produced by *Penicillium islandicum* that has been found frequently in rice [138].

Roquefortine C (Figure 1o) is known for its beneficial toxicity at low concentration (~0.5 mg/kg) present in carbonated beverages, alcoholic drinks, blue cheese [139], and other domestic cheeses [140] but highly toxic at higher (>1.5 mg/kg) concentrations [141].
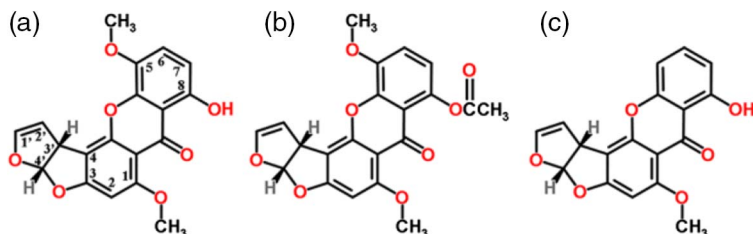
Figure 4. The chemical structure of antileukemic 5-methoxysterigmatocystin (5-MS) derivatives. (a) 5-MS, (b) O-acetyl-5-MS, (c) sterigmatocystin. The ring structure consists of 2 five-membered bisfurano rings and 3 six-membered rings forming the xanthone skeleton

Xanthene derivatives – Sterigmatocystins. Among the xanthene-derivative sterigmatocystin (Figure 4), the mycotoxin 5-5-MS was isolated from an *Aspergillus* sp. strain. Subsequent purification revealed that the active substance 5-MS possessed strong antitumor effects [142]. The antileukemic effect of 5-MS was similar to that of O-acetyl-5-MS (Figure 4b) in tests on leukemia cells, by prolonging their survival over a fourfold dose range. The potency and toxicity of sterigmatocystin (Figure 4c) was about one-half of those of 5-MS and O-acetyl-5-MS. The toxicity and antileukemic effects were low in other 5-MS derivatives, such as O-methyl-5-MS and dihydro-5-MS and absent in 4-3′-furyl-3,8-dihydroxy-1,5-dimethoxyxanthone (iso-5-MS) [142]. The antineoplastic effects of several sterigmatocystins were presented by Bradner's group [15]. This team also revealed that the antitumor activity of the 5-MS parent compound has been associated with the intact bisfurano ring system and with the double bond in the terminal furan ring. The "Janus-faced" bisfurano rings are also present in the carcinogenic aflatoxin.

Mycophenolic acid derivatives. Mycophenolic acid was the first antibiotic synthesized in pure form in 1896 [143], and also the first being resynthesized in 1913 [144]. Mycophenolic acid (NSC-129185) was reported as an antineoplastic agent [145] and subjected to clinical trials in 1972 [146, 147]. Its broad-spectrum antiviral, antifungal, antipsoriasis, and anticancer effects were rediscovered in 1997 [148]. Mycophenolic acid was equally effective when administered by either the oral or i.p. route. The LD50 in mice of mycophenolic acid was high and found to be >1,000 mg/kg after oral and i.p. administration [149]. Mycophenolic acid delayed-release tablets are used as immunosuppressants. FDA prescribing information warns of its embryofetal toxicity during pregnancy associated with increased risks of pregnancy loss and congenital malformations. Nevertheless, the broad-spectrum mycophenolic acid tablets serve as a precedent that toxic mycotoxins with severe side effects could be applied among others against cancer and metastasis. The experimental antineoplastic

activity and preclinical toxicology of mycophenolic acid in combination with either radiation or cyclophosphamide showed a synergistic antitumor response against experimental tumors [150].

Correlation was found between the antitumor and immunosuppressive activities of mycophenolic acid derivatives [151]. A few derivatives that possessed potent antineoplastic activities with less or no immunosuppressive activity turned out to be good candidates for cancer chemotherapy [152]. The antineoplastic properties of 65 analogs and derivatives of mycophenolic acid have shown that none of these compounds were as effective as mycophenolic acid itself [153]. Others who have also studied the anticancer potential of derivatives of myco-phenolic acid have found three chemical modifications that deserve mention: TM-1Z03, TM-1Z04, and TM-1Z13 mycophenolic acids (Figure 5). These compounds were recommended as potential candidates for cancer chemotherapy [153]. As the molecular mechanisms of the antineoplastic effects of mycophenolic acid were not completely understood, *in vitro* assays showed that this compound inhibited endothelial cell and fibroblast proliferation, invasion/migration, and endothelial cell tube formation. The involvement of antiangiogenic signaling of mycophenolic acid contributed to its antineoplastic effect, further stimulated clinical investigations [154] and is expected to initiate further antimetastatic animal studies.

Mycophenolate mofetil (morpholinoethylester of mycophenolic acid), the prodrug of mycophenolic acid, has been widely used not only for the prevention of acute graft rejection but also for the growth inhibition of many tumors. Due to its inhibition of cancer cell proliferation, induction of apoptosis, mycophenolate
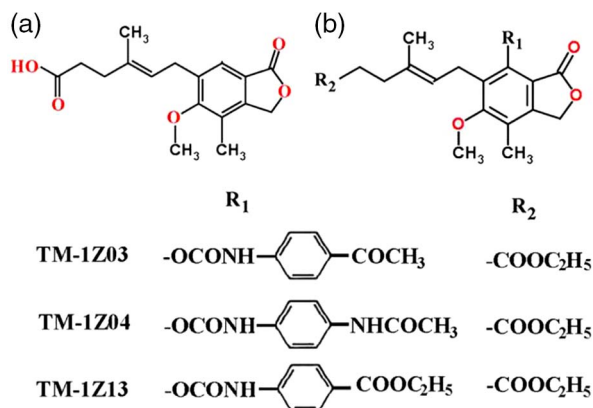
Figure 5. Mycophenolic acid derivatives. (a) Mycophenolic acid, (b) derivatives of mycophenolic acid with antitumor activities

derivatives remained potential candidates for cancer therapy, noting that the underlying molecular mechanisms remained unknown [155]. Besides the immunosuppressive effect of mycophenolate mofetil, it has a strong antineoplastic effect against pancreatic cancer *via* the inhibition of the inosine monophosphate dehydrogenase activity *in vitro* and *in vivo* [156, 157]. Mycophenolate [158] and mycophenolate mofetil [159–161] are useful for the treatment of sarcoidosis and granulomatosis. The inhibition of inosine monophosphate dehydrogenase by mycophenolic adenine dinucleotide analogs and by other mycophenolic acid derivatives as well as their antiproliferative potential was confirmed [162, 163]. However, the immunosuppressive and antitumor effects of mycophenolate and its derivatives based on the inhibition of the pathway of purine nucleotide biosynthesis could impose difficulty in its therapeutic application. Many options for a specific regimen for individual cancer patients could minimize the risk of mycotoxin side effects while enhancing benefits.

Equisetin. This small group of antibiotics produced by *Streptomyces* includes equisetin, an N-methylserine-derived acyl tetramic acid produced by a number of *Fusarium* species with antibiotic and cytotoxic activities [164]. Equisetin also isolated from the fungus *Fusarium heterosporum* and an enantiomeric homolog of equisetin from *Phoma* sp. were effective inhibitors of the HIV-1 integrase *in vitro*. Two additional analogs, the decalin derivative, integric acid, and oteromycin were reported to inhibit HIV-1 integrase [165]. As antineoplastic and antiviral activities of antibiotics are often associated [166], the antitumor potential of equisetin should not be neglected.

Marine-derived mycotoxins

Cytotoxic agents were defined as poisonous compounds that may become potential anticancer drugs when they display: (a) selectivity for cancer cells, (b) activity against multidrug-resistant cancer cells, and (c) preferential non-apoptotic cell death mechanisms against cancer cells [167]. This general distinction concerns not only fungal toxins but also anticancer agents of different origin.

In the past 50 years, several compounds of marine origin led to drugs mainly in the area of cancer therapy [168]. More than 1,000 marine fungal-derived metabolites have been reported. Despite their absence in the current clinical application, dozens of them have been classified as potential chemotherapy candidates because of their anticancer activity [167]. Toxins are derived from the marine fungus *Aspergillus terreus* and grown under various culture conditions generate environmentally induced changes in metabolite expression. Differences can be used to guide the discovery of new bioactive molecules [169]. There is no doubt that some of the marine-derived fungal metabolites could

become actual anticancer agents, suggesting that marine-derived metabolites have a bright future.

Aurantosides. These are cytotoxic tetramic acid glycosides isolated from marine *Theonella* species. Aurantosides exhibit antifungal activity against *Aspergillus fumigatus* and *C. albicans* [170, 171] but are not regarded as potential antitumor agents.

Phenyl-cytochalasins. New cytochalasins derived from the marine fungus *Arthrinium arundinis* showed cytotoxicity against several cell lines with IC50 values ranging between 1 and 50 μM [172]. Z_7, Z_8, Z_9, cytochalasin E and K are phenyl derivatives isolated from the marine-derived fungus *Spicaria elegans* [173]. Phomopchalasins with polycyclic-fused rings exert cytotoxic, anti-inflammatory, and anti-migratory activities [174].

Structure and function relationship of mycotoxins

The three-dimensional (3D) structure is expected to define not only the size and shape but also the function of mycotoxins. Unfortunately, mycotoxins have too many functions and in spite of functional similarities, their structures may be quite different.

For exact 3D structural measurement *in silico* models could be used, but these calculations should consider covalent, electrostatic, van der Waals, and steric interactions of structural elements of mycotoxins. Due to the complexity of these interactions, the use of such computer models remained limited. We have compared 2D structures with particular attention to ring structures and to the most often occurring functional groups in anticancer mycotoxins.

From the data contained in the figures and tables, the structure–toxicity relationship led to the following general conclusions:

1. *Heteroatoms in ring structures.* Most of the mycotoxins contain oxygen heteroatoms in saturated, unsaturated, or aromatic rings. The distribution of oxygen heteroatoms in the rings of mycotoxin structures is summarized in Table 2. The majority of compounds (78%) contained oxygen. In oxygen-bearing mycotoxins, one oxygen was present with 35%, two oxygens with 42%, three oxygens with 19%, and four oxygen with 4% frequency. Nitrogen was half as frequently present in 19% of the mycotoxins with 1–5 N atoms. Sulfur as heteroatom was present only in gliotoxin. Fumonisin B1 and the enolic form of penicillic acid were the only aliphatic compounds without ring structures. It was realized only in the late 1990s that hundreds of thousands of compounds that were synthesized lacked "complexity" associated with bioactive native natural compounds including chiral centers, heterocyclic substituents, and polycyclic structures. After this recognition,

the combinatorial chemistry was favored that has revolutionized the development of active compounds [175]. In agreement with these recognitions, those mycotoxins were selected and used jointly, which possessed the attributes of complexity. The application of new techniques and methodologies helped to develop compounds into active antitumor agents [176].

2. *Presence of reactive oxygen atoms*. All mycotoxins summarized in Table 2 contain oxygen. The cumulative presence of oxygen atoms (213 oxygens in 32 mycotoxins), especially the highly reactive three atom ring epoxide could be responsible for generating oxidative stress. However, the generation of extremely aggressive ROS should be avoided [177], exemplified by trichothecenes, where these ROS-generating species did not improve the antineoplastic activity of mycotoxins [18].

3. *Functional groups*. Among the oxygen-containing functional groups, the ratio of keto (40%) and hydroxy (47%) groups was the highest; carboxy groups represented only 13% of the oxygen-containing functional groups. The extremely high number (21) of hydroxy groups in aflastatin relative to the two keto moieties could be accounted for by the inhibitory effect of aflatoxins. These groups could be responsible for the cytotoxic and antineoplastic effects of mycotoxins in general. The frequency of amino and imino groups together was only about 5% relative to other oxygen-containing functional groups (Table 2).

As far as their carcinogenicity to humans is concerned, only aflatoxins belong to the most carcinogenic IARC 1 category (Figure 6a). Based on the partial structural similarity of aflatoxin B1 and 5-MS (boxed in Figure 6a and 6b) and similarities in the rest of their structures, 5-MS appears to be the second most dangerous mycotoxin belonging to the possibly carcinogenic IARC 2B category. The cytotoxicity in other IARC 2B carcinogens such as ochratoxins and fumonisins is definitely contributed by keto, hydroxy, and the cumulative presence of carboxy groups (Figure 6c and 6d). Mycotoxins belonging to higher IARC numbers are not classifiable as to their carcinogenicity to humans. Major mycotoxins belonging to the non-carcinogenic IARC 3 category are nivalenol, deoxynivalenol, patulin, T-2/HT-2 toxins, zearalenone [4].

The structure–toxicity relationship of the best studied and one of the largest group of mycotoxins known as trichothecenes [178] allowed the following generalization:

– *Epoxy ring*. The 12,13-epoxy ring is the most important structural feature providing high toxicity to trichothecenes.

– *Ring structure of trichothecenes*. The double bond between C9–C10 and the 12,13-epoxide ring are essential structural features for the trichothecene toxicity. Removal of these groups results in the complete loss of toxicity.
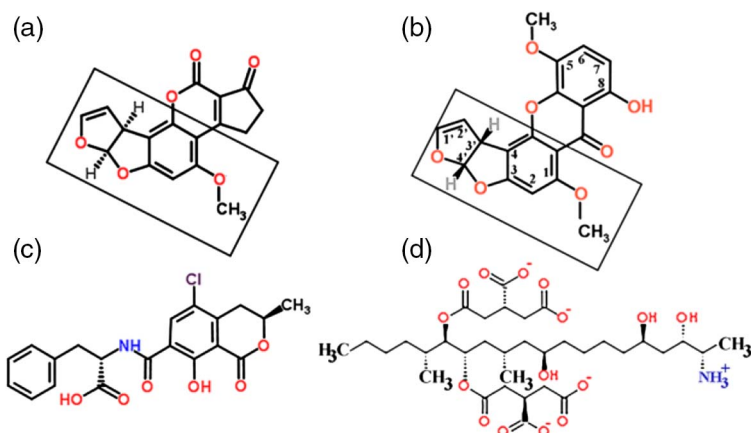
Figure 6. Structure and IARC classification of major mycotoxins. (a) aflatoxin B1 – IARC I category, (b) 5-methoxysterigmatocystin (5-MS) – IARC 2B category, (c) ochratoxin A – IARC 2B, (d) fumonisin B1 – IARC 2B. Identical parts of aflatoxin B1 and 5-MS are boxed

Nitrogen is absent, but as referred to above, it significantly does not affect toxicity.

– *Hydroxy group* at C3 enhances trichothecene toxicity, while this activity decreases gradually when C3 is substituted with either hydrogen or an acetoxy group. The presence of a hydroxy group at C4 promotes slightly lower toxicity than an acetoxy group at the same position. The presence of hydroxy and hydrogen groups on C15 decreases the trichothecene toxicity in comparison with an acetoxy group attached to this carbon. The substitutions point to the importance of a high number of oxygen atoms and to the defined positions of hydroxylations.

– *Frequency of functional groups*. The most frequently occurring substituent in both type A and type B trichothecenes is the hydroxy group followed by acetyl and keto groups.

The large number and the general structural features of trichothecenes allow postulating that the chemical structure of trichothecenes has the molecular capability to be tailored for optimal antitumor and antimetastatic purposes. In antimetastatic rodent tumor models, trichothecenes are not to be ignored but recommended.

Relationship of Mycotoxins to Metastatic Studies

Transportation of tumor cells from the primary tumor to another part of the body may take place through the bloodstream (hematogenous metastasis) or

through the lymphatic system (lymphoid metastasis). Earlier views favored the hematogenous tumor spread of metastasis. A great number of circulating tumor cells are quickly killed by the immune system, by hemodynamic forces and apoptosis, whereas only a small fraction of cells turns to metastasis [179] or, if extravasated, capable of forming micrometastases. The current view implies that tumor growth and metastasis are generated by a complex molecular mechanism. We have described the metastatic spread of tumor cells from abdominal primary tumors of rats to parathymic lymph nodes (PTNs) [180] that involve:

1. Delayed angiogenesis. The primary tumor necrosis develops in the inner part of the primary tumor, and limited angiogenesis drives the blood and the tumor cells to the outer part of the tumor and to interstitial fluid [181].
2. Vascular disruption at the periphery aggressively promotes the release of tumor cells from the primary tumor [182].
3. The spread of tumor cells (solid and leukemic) released by abdominal tumors of rats is directed toward the PTNs, which turned out to be the sentinel lymph nodes [183].
4. Our rat metastatic tumor model has shown that delayed angiogenesis in the primary tumor necrotized its inner part and the increased blood pressure channeled through the interstitial fluid of the outer part of the tumor causing disruptions and the release of blood and tumor cells into the neighboring tissues or cavities. In turn, the broken away blood cells along with the released tumor cells were either engulfed by the tissue macrophages, drained to the lymphatics, to the nearest sentinel lymph node, and lymph node chain [180, 184] or could spread through the interstitial and tissue fluids to other thoracal tissues and lymph nodes.

The metastatic spread from the abdominal primary tumor to thoracal and mammary lymph nodes is summarized in Figure 7. Hemangiogenesis and ruptures at the outer part of the primary tumors are opposed by the necrotization of the inner part of the abdominal tumor. Ruptures of hemorrhagic regions in the primary tumor release tumor cells to the peritoneum. Tumor cells cross the diaphragm and metastasize to the thoracal lymph node chain. Human internal mammary lymph nodes (IMNs) harbored inside the thymic capsule correspond to rodent PTNs, located outside the thymic sheath. Tumor cells generate peritoneal (liver) or retroperitoneal (kidney) primary solid tumors and the presence of abdominal leukemia cells may initiate leukemia. Migration of tumor cells from the disrupted surface of the abdominal primary tumors of liver, spleen, and kidney takes place through the lymphatic anastomoses of the diaphragm to PTNs (IMNs) or directly from the subdiaphragmatic lymphatics to the breast [184].
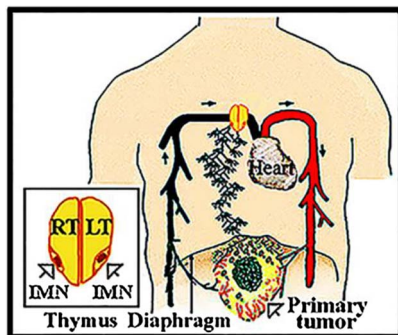
Figure 7. Tumor cells released by abdominal tumors metastasizing to thoracal lymph nodes. The metastatic spread of tumor cells released by abdominal primary tumors (e.g., liver and kidney). Lymphatic passage of tumor cells through the diaphragm to thoracal and mammary lymph nodes. Human IMN, internal mammary lymph node = rodent PTN. Modified with permission [184]

Strategy of Using Mycotoxins Against Metastasis

Metastatic rat model

Similar to the murine model [126], the metastatic rat tumor model is suitable for the early detection of primary tumors and metastasis [180] and could be used to test antineoplastic mycotoxins. The review has compared those mycotoxins that could be potential candidates against the metastatic spread of tumor cells released by abdominal primary tumors. Oxidative stress-induced cell death *via a* mitochondrial-dependent burst of ROS [185] will be generated by mycotoxins and tested in animal tumor experiments. Broad-spectrum mycotoxins with strong antitumor and immune suppressive control, along with the trichothecene analogs [83, 84] will be first tested to compare their efficacy in an *in vivo* rodent model. The benefits of analogs will have to be balanced against their potential negative side effects.

In vivo effect of mycotoxins against rat tumors

Selected mycotoxins will first be tested *in vitro* on cells released by the primary tumor, then at the level of cytomics cell system (cytome) at the single-cell level followed by time-lapse microphotography [186]. Furthermore, *in vivo* studies will clarify whether or not i.p. administered mycotoxins will be able to prevent the metastatic spread of abdominal primary tumor cells by killing them before they would reach their metastatic target in the rodent PTNs. These results

will provide critical data related to the spread of tumor cells to gain time for therapy and prevention of metastasis.

This antimetastatic strategy aims to utilize a mycotoxin-based suicide therapy *via* the oxidative stress-induced cell death by initiating a mitochondrial-dependent burst of ROS [187]. For this purpose, not only mycotoxins could be utilized as single agents, but also antineoplastic responses combined with other chemotherapeutical drugs as potential amplifiers of the antineoplastic activity against experimental tumors. Transformations to closely related derivatives of mycotoxin structures indicate that chemical tailoring may lead to optimal solutions or at least to a *modus vivendi* regarding the cytotoxicity and reduction from undesirable to tolerable side effects of mycotoxins. Of particular importance are potential synergisms between mycotoxins with regard to both their anticarcinogenic efficiency and carcinogenic attributable risk, pointing to the importance of future studies of mycotoxins [188].

Conclusions

After matching the functional demand with the structural design, for animal experiments against metastatic growth, none of the mycotoxins have been found to be directly applicable. However, more potent mycotoxin analogs with fewer side effects could result in efficient antineoplastic, immunosuppressant combinations. This necessitates the selection of few compounds or a diverse group of mycotoxins that possess the ability of high cytotoxicity combined with antineoplastic, immunosuppressive potential. Trichothecenes have been preselected as they represent one of the most diverse and controversial groups due to the wide variety of biological activities depending on the functional groups present in the tricyclic skeletons. Among the varieties of sesquiterpenoid trichothecenes, the structure–activity relationship of each ring of synthetic deserves further exploration. Anguidines widen the group of trichothecene mycotoxins also exerting antitumor effects. The best-known teratogenic trichothecene anguidine with its hypotensive effect was tested but poorly tolerated by patients, leading to its unfavorable reputation. Ethical consideration speaks against the use of mutagenic agents, which may cause birth defects or long-term benign tumors, but are unlikely to threaten seriously old metastatic patients. Other trichothecenes and fumonisins that affect testicular and ovarian functions are probably not worth to be improved and used as antitumor agents. Similarly, before clarifying the cytotoxicity and *in vivo* side effects, it would be too early to test other less known mycotoxins for their antitumor potential in animal models.

The improvement of other potential antileukemic derivatives of 5-MS and mycophenolic acid derivatives deserve to be mentioned. Of the recently described 127 mycotoxin combinations, aflatoxin–fumonisin, deoxynivalenol–zearalenone, aflatoxin–ochratoxin, and fumonisin–zearalenone are the best-known ones [189]. These and related combinations could be tested as antitumor agents in animal models. It is expected that further studies help to reveal structure–toxicity relationship of mycotoxins and will contribute to the better understanding of mycotoxins, risk assessment, and antitumorigenic potential and used for the therapy of metastasis.

As part of this strategy, the immunosuppressant properties of mycotoxins could also be used to prevent tumor growth and metastasis [190], but the major focus of our future *in vivo* rodent experiments remains directed toward the *in vivo* effect of antineoplastic mycotoxins.

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Conflict of Interest

The authors have no conflict of interest to declare.

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