Published in final edited form as:

Med Res Rev. 2015 September ; 35(5): 937–967. doi:10.1002/med.21348.

Towards a Cancer Drug of Fungal Origin

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Abstract

Although fungi produce highly structurally diverse metabolites, many of which have served as excellent sources of pharmaceuticals, no fungi-derived agent has been approved as a cancer drug so far. This is despite a tremendous amount of research being aimed at the identification of fungal metabolites with promising anticancer activities. This review discusses the results of clinical testing of fungal metabolites and their synthetic derivatives, with the goal to evaluate how far we are from an approved cancer drug of fungal origin. Also, because *in vivo* studies in animal models are predictive of the efficacy and toxicity of a given compound in a clinical situation, literature describing animal cancer testing of compounds of fungal origin is reviewed as well. Agents showing the potential to advance to clinical trials are also identified. Finally, the technological challenges involved in the exploitation of fungal biodiversity and procurement of sufficient quantities of clinical candidates are discussed and potential solutions that could be pursued by researchers are highlighted.

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Keywords

Fungal metabolites; clinical trials; drug resistance; anguidine; aphidicolin; rhizoxin; fumagillin; illudin S; phenylahistin; wortmannin

1. Introduction

1.1. Recent Advances in the Treatment of Cancer

Although cancer remains a devastating diagnosis, advances in cancer biology and biotechnology have recently led to the successful development of a number of drugs that improve survival and quality of cancer patients' lives.¹ Thus, during the last 5 years, delay-adjusted cancer incidence rates in the USA declined slightly in men (by 0.6% per year) and were stable in women, while cancer death rates decreased by 1.8% per year in men and by 1.4% per year in women.² Among all the strategies used to combat various types of cancers, cytotoxic agents remain powerful weapons.³ In addition to these, recent additions include, among others, targeted therapies,⁴ therapies based on cancer epigenetics,⁵ photodynamic therapy,⁶ immunotherapy⁷ and antiangiogenic agents.⁸

1.2. The Role of Natural Products in Cancer Drug Discovery

Newman, Cragg and coworkers⁹⁻¹¹ have provided comprehensive reviews emphasizing the pivotal role of natural products as anticancer agents. They reported that in the cancer area, of the 175 small molecules utilized clinically (1940s to date), 131 (~75%) are other than synthetic, being either natural products or directly derived therefrom.¹⁰ Accordingly, Qurishi et al.¹² state that even today, in the presence of massive numbers of agents from combinatorial libraries, compounds from natural sources are still in the forefront of cancer chemotherapeutics as sources of drugs. The group of M. Diederich^{13,14} also recently reviewed the significant potential of natural compounds as anticancer agents.

Natural products derived from bacteria and plants play a leading role in cancer drug discovery, which is demonstrated by a large number of approved anticancer agents derived from these sources. Such drugs include effective chemotherapeutic agents such as doxorubicin, daunomycin, mitomycin C, bleomycin (all obtained from *Streptomyces*), and etoposide, teniposide, irinotecan, topotecan, paclitaxel, docetaxel, vincristine, etc. (obtained or derived from plant-based natural products). Fungi-derived natural products have been an excellent source of pharmaceuticals as well. However, fungal biodiversity has been only partially exploited, because only an estimated 5% of fungi have been cultured in laboratories.¹⁵ However, fungi would offer an enormous source of novelty if the constraints of their isolation and culturing could be overcome.^{16,17} Fungi produce metabolites belonging to highly diverse structural classes, including aromatic compounds, amino acids, anthracenones, butanolides, butenolides, cytochalasans, macrolides, naphthalenones, pyrones, terpenes, among others.¹⁸⁻²⁰ Surprisingly however, no fungi-derived agent has been approved as an anticancer drug so far, despite a tremendous amount of research being aimed at the identification of fungal metabolites with promising anticancer activities.

1.3. Advantages and Limitations of Fungi as a Source of Anticancer Agents

Despite a large number of anticancer hits identified in collections of compounds isolated from fungi, there are a number of technological, biotechnological and physiological factors that are detrimental for applicative research or industrial development of fungal metabolites. However, strategies to overcome these limiting factors and allow fungi to express their “real” metabolic potential, probably in some cases far superior to that of bacteria and plants, are currently being only partially exploited. Basically, procurement and supply of materials of botanic or bacterial origins are easier to accomplish, when compared to fungal fermentation, as plants can be easily grown and harvested as a crop, and bacteria can be grown more easily than fungi in bioreactors by liquid shaken fermentation. Thus, the amount of a metabolite produced by a fungus and its cost of production could really represent a constraint for the advancement to clinical studies. Indeed, often promising metabolites are produced by fungi in laboratory in milligram amounts, generally sufficient only for the preliminary anticancer bioassays, or are commercially available as expensive biochemical reagents. For example, aphidicolin and phenylahistin, promising anticancer agents discussed later in the review, were isolated in milligram quantities per liter of a liquid culture of *Nigrospora sphaerica*²¹ and *Aspergillus utus*,²² respectively. Other promising metabolites are often isolated in even lower amounts.²³⁻²⁵

All or almost all fungal metabolites are initially obtained in the laboratory using traditional methods, i.e. growing the fungus in purity on liquid substrates having simple and mineral defined composition, in order to more easily isolate and identify substances produced by the fungi. In other cases, fungi are grown in static conditions, or even on solid substrates. However, little attention has been given to the production aspects, and only a limited number of secondary metabolites have been really investigated for a possible scale-up in bioreactors.²⁶ The most used bioreactors are liquid shaken, but unfortunately the highly branched fungal mycelia could cause many problems in these conventional agitated-tank fermentors. The formation of mycelial clumps and pellets in the fermentor not only limits mass transfer, but also increases the broth viscosity, reduces oxygen transfer, and causes difficulty in mixing. In order to solve these problems, cell immobilization methods have been employed to control cell morphology and achieve higher cell density and productivity in several filamentous fungal fermentations.^{27,28} In general, the immobilization of filamentous mycelia can be achieved either by cell attachment via adsorption to a surface or self-immobilization by forming cell pellets.

Greater attention could also be devoted to solid-state fermentation (SSF) processes, i.e. the growth of microorganisms on moist solid substrates in the absence of free-flowing water. This can be carried out both on natural substrates (the most used), which serve as a support and a nutrient source (typically starch- or ligno-cellulose-based agricultural products or agro-industrial sources), and on inert supports (e.g. perlite, polyurethane foam, vermiculite) impregnated with a liquid medium.²⁹⁻³¹ Even the use of new or low-cost substrates can improve the production capacity of fungi or also reduce costs. Using processing waste from the food industries may also offer fungi a huge pool of compounds for their metabolism, potentially resulting in completely new sets of unpredictable compounds.

Another element critical for success in microbial natural product-based discovery, which has received relatively little consideration, is the manipulation of nutritional and environmental factors promoting secondary metabolite biosynthesis. It has also been noted that minor variations in the growth conditions or in nutrient availability can strongly impact the quantity and diversity of fermentation products.^{32,33} The deliberate elaboration of cultural parameters to augment the metabolic diversity of a strain has been called the OSMAC (one strain, many compounds) approach.³⁴⁻³⁶ The advent of new technologies, high throughput bioassays and analytical methodologies provides the opportunity to create new and unnatural environmental and nutritional conditions that can change the metabolite production in an almost unlimited and unpredictable manner. Temperature, light, quantity and quality of micro- and macronutrients available, structure of the substrate, shaken conditions, gas availability etc., can significantly affect the ability of fungi to produce secondary metabolites, both in quantitative and qualitative terms. For example, Bode et al.³⁴ isolated more than 100 compounds from more than 25 structural classes, from six different microbes by altering culture conditions. In this work, the fungus *Sphaeropsidales* sp., which synthesizes the antifungal spiro-bisnaphthalene-cladosporone-bis-epoxyde, made eight new and six known spironaphthalenes when grown under varied conditions, as well as new bis-naphthalenes and a rare macrolide, when grown in the presence of enzyme inhibitors such as tricyclazole.³⁴ Furthermore, *Phoma exigua* var. *heteromorpha* produced deoxaphomin (a 13-cytochalasan), several 14-cytochalasans (deoxaphomin, cytochalasin A, B, F, T, 7-O-Acetyl-CB) and many 24-cytochalasans (cytochalasins Z1–Z5) on solid medium. When grown in liquid culture it produced ascochalasin (13-cytochalasan), deoxaphomin, cytochalasin A and B (all 14-cytochalasans), together with cytochalasin U and V (15- and 16-cytochalasans, respectively). Only three compounds out of fourteen were produced in both cultural conditions,³⁷ highlighting the importance of the conditions in this regard.

Filamentous fungi are rarely fermented below the flask or tube scale, and the extent to which secondary metabolite production can be scaled down is generally unknown. Nutritional or environmental arrays could be applied to identify organisms and conditions in which they would be more able to produce secondary metabolites, as a first step in microbial screening, resulting in screening populations enriched in biological activity.³²

Another approach to exploit the metabolic potential of cultivatable microbes is mixed fermentation, where the presence of neighbouring microbes may induce secondary metabolite synthesis. Mixed fermentation can result in increased biological activity in crude extracts, increased yields of previously described or previously undetected metabolites, analogues of known metabolites resulting from combined pathways and, importantly, induction of previously unexpressed pathways for bioactive constituents.³⁸

The sequenced genomes of fungal species and the identification of the biosynthetic pathways have opened the door to engineering novel analogues of many structurally complex metabolites. Biotransformation relies on the inactivation of a biosynthesis gene followed by a comparative metabolic profile analysis of the mutant and the wild type, e.g. by HPLC or LCMS. For instance, this strategy was successfully employed by Chiang et al.³⁹ on *Aspergillus nidulans* for the production of several novel emericellamide-related compounds, whereas the disruption of *F. sporotrichioides* Tri11, a gene encoding a

cytochrome P-450 monooxygenase, led to the accumulation of four trichothecenes not observed in cultures of the parent strain.⁴⁰

Other genetic strategies to improve natural products biosynthesis in the industrial setting rely upon iterative rounds of random mutagenesis and empirical screening to achieve titer improvements.¹⁵ New strategies can complement the traditional methods to increase the overall efficiency and lower the costs of the commercialization process. The development of molecular microbiology and recombinant DNA technology has led to a number of strategies for rational strain improvement known collectively as metabolic engineering.^{41,42} The hierarchical structure of secondary metabolite regulation offers two distinct strategies for engineering: (1) manipulating global regulators to increase production of secondary metabolites; (2) targeting pathway specific regulators for titer increase of a particular compound of interest. It should be noted that global regulators may also function across different producers.⁴³

1.4. Resistance of Cancer Cells to Chemotherapy

As emphasized by Holohan et al.,⁴⁴ resistance to chemotherapy and molecularly targeted therapies is a major problem facing current cancer research. In addition, as reviewed by Vadlapatha et al.,⁴⁵ it appears that acquisition of multidrug resistance (MDR) represents one of the major impediments to successful chemotherapy. In addition to the MDR phenotype, there exists a large panel of other drug resistance mechanisms in cancer cells,⁴⁶ including the cancer cell resistance to pro-apoptotic stimuli.⁴⁷⁻⁴⁹ Various strategies have thus been developed to combat, at least partly, the drug resistance of cancer cells.⁵⁰⁻⁵² In this regard, targeting of epigenetic features could represent a promising opportunity,⁵³ including the use compounds of natural origin.⁵⁴ The application of small molecules to induce non-apoptotic cell death is also a viable possibility to overcome drug resistance in cancer cells, especially those displaying resistance to apoptosis.⁵⁵ Fungal metabolites represent an important source of compounds capable of overcoming these resistance mechanisms and warrant their extensive exploration as anticancer agents with significant clinical benefits against resistant tumors and/or their metastases.⁵⁶

Revently, we reviewed various chemical structures and mechanisms of action of fungal metabolites as potential anticancer agents.⁵⁶ The potential of macroscopic mushrooms as a source of compounds with anticancer activity has also been recently reviewed.⁵⁷ The current review explores the question of how far we are from a marketed anticancer agent derived from a fungus.

2. Fungal Metabolites and their Synthetic Analogues in Cancer Clinical Trials

2.1. A Detailed Description

A number of fungal metabolites and/or their analogues have progressed to various stages of cancer clinical trials. The structures of these compounds are shown in Figure 1 and a summary of the clinical trial designs and outcomes for these compounds is provided in Table 1.

Anguidine—Anguidine (Figure 1), also known as 4,15-diacetoxyscirpenol, is a known mycotoxin belonging to the group of trichothecenes produced by several *Fusarium* species, including *F. roseum*, *F. sambucinum*, *F. tricinctum*, *F. equiseti*, *F. lateritium*, *F. graminearum*, *F. semitectum*, *F. sulfureum*, *F. diversisporum*, *F. scirpi*, and *Giberella intrigans*.^{18,20} Anguidine irreversibly blocks protein synthesis by inhibiting the protein chain initiation through degradation of polyribosomes. Early preclinical studies at the National Cancer Institute (NCI) revealed >100% life-span extension of P388 leukemia-bearing mice with chronic i.p. administration of anguidine.⁵⁸ However, based on a broad Phase II study conducted at the M. D. Anderson Hospital and Tumor Institute and the University of Kansas (the Southeastern Cancer Study Group) from November 1977 to June 1981 (Table 1), no meaningful activity was found in six tumor categories and the conclusion was made that anguidine did not warrant additional evaluation for the treatment of solid tumors.⁵⁹⁻⁶⁴

Aphidicolin—Aphidicolin (Figure 1), is a diterpenoid metabolite produced by different fungi including *Cephalosporium aphidicola*, *Nigrospora sphaerica* and *Harziella entomophilla*.¹⁸ Its structure was determined using chemical and spectroscopic data^{65,66} and later confirmed by its stereocontrolled synthesis.⁶⁷ More recently, aphidicolin was also isolated from *Phoma* sp. 7210 associated with *Aizoon canariense*.⁶⁸ This fungal metabolite is a specific inhibitor of DNA polymerases α and δ . Inhibition of DNA polymerases α and δ by aphidicolin is reversible, and for this reason, the compound has been widely used as a synchronizing agent in experimental systems.⁶⁹ Its water-soluble analogue aphidicolin glycinate underwent Phase I clinical trials that established a dosage and 24 hour continuous infusion as the recommended schedule for clinical evaluations of aphidicolin glycinate as a synchronizing agent, or in combination with cisplatin.⁶⁹

Rhizoxin—Rhizoxin (Figure 1), is a phytotoxic 16-membered macrolide, initially isolated from the cultures of the fungus *Rhizopus chinensis*, the causal agent of rice seedling blight. Its structure was determined by spectroscopic and chemical experiments involving the preparation of several key derivatives.⁷⁰ Successive studies proved this potent antimicrobial polyketide not to be produced by the fungus itself, but rather by endosymbiotic bacteria belonging to the *Burkholderia* genus, among which the best producer appeared to be *Burkholderia rhizoxina*.³⁶ More recently, a combination of genetic and chemical studies allowed to ascertain that the macrolide is first epoxidized by the bacteria cytochrome P450 monooxygenase RhiH, and that the 2,3-oxirane ring is then introduced by the fungal host to specifically tailor the rhizoxin scaffold.⁷¹ Of interest is that the additional epoxide moiety substantially increases phytotoxic potency. Successively, the large-scale fermentation of this bacterium allowed producing and identifying a large number of rhizoxin natural analogues.

Rhizoxin is known to bind to tubulin at the vinca site and inhibit microtubule assembly. It was shown to possess cytotoxicity against a variety of human tumour cell lines and xenograft models.⁷² After Phase I clinical trials provided the maximum tolerated dosage of the compound and revealed its generally low level of systemic toxicity,⁷³ Phase II clinical trials were initiated by the EORTC Early Clinical Trials Group with the focus on “difficult to treat” cancers. These included melanoma, squamous cell head and neck cancer and

NSCLC. It was concluded in the melanoma trial that, although patients were tolerant to the dosage administered, no significant improvement in patient conditions was observed.⁷⁴ With respect to the squamous cell head and neck cancer trial, rhizoxin was shown to have minor activity, with two partial responses being noted.⁷⁵ Lastly, and more promising, in the NSCLC trial, the researchers observed better results. This included 4 partial responses and almost half of the patients treated showed stabilization of the disease condition. The conclusion in this particular trial was that rhizoxin showed activity as a single agent, and its application to NSCLC treatment was thus an attractive proposition for further evaluation.⁷⁶

Fumagillin—Fumagillin (Figure 1), is a complex metabolite first isolated from the liquid culture of *Aspergillus fumigatus* strain H-3.⁷⁷ Its structure was assigned based on chemical studies which included the preparation of a number of key derivatives.⁷⁸ Of interest was that biosynthetic studies showed that fumagillin in part arises by a terpene route and in part by the acetate pathway.⁷⁹ Fumagillin was also produced by other species of *Aspergillus*, including *A. flavus* and *A. parasiticus*.⁸⁰ Recently, a chlorinated derivative of fumagillin (ligerine) was isolated from a marine-derived *Penicillium* strain, showing strong inhibitory activity against an osteosarcoma cell line.⁸¹ Synthetically derived fumagillin analogues, TNP-470, PPI-2458 and CKD-732 (Figure 1), have been evaluated in human cancer clinical trials. Together with fumagillin, these synthetic analogues disrupt tumor vasculature by targeting the enzyme methionine aminopeptidase type 2, which cleaves the *N*-terminal methioninyl residue of newly synthesized proteins.⁸² Although promising results for TNP-410 were reported in patients with prostate cancer, NSCLC and melanoma (Table 1), neurotoxicity was observed to be an important side-effect, seemingly limiting its clinical usefulness. Understanding the mechanism of neurotoxicity and developing strategies to overcome this dose-limiting side-effect would be required before this agent could be widely used in a clinical setting. The newer analogues, PPI-2458 and CKD-732, appear to alleviate the neurotoxicity issues associated with TNP-470, but their therapeutic effects remain to be demonstrated in the clinic.

Illudin S—Illudin S (Figure 1), also known as lampterol, is a sesquiterpenoid firstly isolated as an antibacterial metabolite from *Clitocybe illudens*, together with its analog illudin M.⁸³ The structure of the sesquiterpene was later assigned by spectroscopic and chemical methods, confirming that illudin S and lampterol, isolated from *Lampteromyces japonicus*,^{84,85} were identical. Illudin S was also produced from *Omphalotus olearius*, *Colybia nivalis*, *Favolaschia* sp. and *Pterula* sp., when grown on natural substrates.⁸⁶ The anticancer activity of this natural product has been shown to stem from the alkylation of DNA, RNA and proteins.⁸⁷ Of additional interest is that there are reports of 19 clinical trials which have been conducted with its semisynthetic analogue irofulven (Figure 1) - some of the noteworthy ones are provided in Table 1. Overall, although evidence of antitumor activity was observed, this agent appears to have a narrow therapeutic index, which will limit its use in the clinic. In particular, strong retinal toxicity observed with this agent is disturbing and often disabling.⁸⁷

Phenylahistin—Phenylahistin (Figure 1), is a fungal diketopiperazine metabolite, consisting of phenylalanine and isoprenylated dehydrohystidin, which was isolated from

Aspergillus ustus as racemic mixture.²² Later, the enantiomers were separated by chiral HPLC and their structure assigned by physical and spectroscopic data.⁸⁸ Of interest to this review, was that (-)-phenylahistin was found to exhibit antitumor activity *in vitro* against eight tumor cell lines (A431 dermal, A549 lung, Hela ovary, K562 leukemia, MCF7 breast, TE-671 CNS, WiDr colon) with IC₅₀ values ranging from 0.18 to 3.7 μ M. However, the (+)-enantiomer exhibited 33-100 $\times$ less potent activity. (-)-Phenylahistin also showed antitumor activity against P388 leukemia and Lewis lung carcinoma cells *in vivo*.⁸⁸ This natural product has been shown to have potent vascular disrupting properties through binding to the colchicine site on beta-tubulin.⁸⁹ Currently, its semisynthetic analogue NPI-2358 (plinabulin, Figure 1) is in clinical trials involving patients with NSCLC (Table 1). A Phase I study of NPI-2358, in combination with docetaxel, revealed this combination to be quite tolerable with promising indications of antitumor activity. Currently, this combination is being assessed in a randomized Phase II clinical trial in previously treated patients with advanced and metastatic NSCLC.⁸⁹

Wortmannin—Wortmannin (Figure 1), is a furanosteroid metabolite isolated from *Penicillium wortmannii*. After an initial assignment,^{90,91} its structure and absolute stereochemistry confirmed by X-ray analysis.⁹² Wortmannin was also isolated from *Fusarium torulosum*⁹³ and *Trichoderma* sp. MFF-1.⁹⁴ This fungal metabolite is an inhibitor of phosphatidylinositol 3' kinases (PI3Ks) and phosphatidylinositol 3' kinase-related kinases (PIKKs), such as DNA-dependent protein kinase.^{95,96} Preclinical studies revealed promising radiosensitizing activity associated with this metabolite. However, its clinical translation was limited by high toxicity, poor stability and low water solubility. Although over the years, a large number of analogues have been developed to overcome wortmannin's poor pharmacokinetic properties,^{97,98} today only one, PX-866 (Figure 1), remains under clinical evaluation (Table 1).

2.2. In Summary: Are we close to an approved drug?

The data reported in Table 1 indicates that the majority of agents tested in human clinical trials show little or no antitumor activity discouraging their further investigation. This appears to be the case with anguidine, rhizoxin and irofulven. Neurotoxicity, nonmyelosuppression, poor water solubility, retinal toxicity, insomnia, confusion have been reported as dose-limiting toxicities leading to narrow therapeutic indexes for the majority of agents in Table 1.

However, two compounds are promising in terms of toxicity/efficacy ratio, namely NPI-2358 (plinabulin), a synthetic analogue of halimide (Figure 1), which targets the colchicine binding site on beta-tubulin and is a vascular disrupting agent, and PX-866 (a synthetic analogue of wortmannin, Figure 1), which is a PI3K and PIKK inhibitor (Table 1). However, only Phase I data have been reported in 2012 for these two compounds and their actual efficacy in cancer patients still remains to be demonstrated (Table 1). It must be noted that PX-866 is presently undergoing Phase II trials involving patients with recurrent or metastatic squamous cell carcinoma of the head and neck (NCT01252628, PX-866-003), as well as advanced melanoma (combination of PX-866 and vemurafenib, NCT01616199, PX-866-007). Finally, there is an ongoing, but closed, Phase II trial investigating the

efficacy of PX-866 in glioblastoma multiforme patients (NCT01259869, I204). Thus, the efficacy and the therapeutic ratio of PX-866 and NPI-2358 in cancer patients remain to be demonstrated.

3. Fungal Metabolites and their Synthetic Analogues Evaluated *in vivo* in Mouse Models of Human Cancer

3.1. A Detailed Description

Although *in vitro* studies are useful, for example to decipher the mechanism of action of a given compound of interest, we limited our current review of the data published in the literature to *in vivo* studies because *in vitro* studies are generally not predictive of the behaviour of a given compound in clinical situations, especially with respect to cancer patients. The structures of compounds tested in various *in vivo* cancer models, predominantly in mice, are shown in Figure 2 and the summary of the results is provided in Table 2.

Triornicin—Triornicin (Figure 2), is a siderophore isolated from the fungus *Epicoccum purpurascens*. Its structure, closely related to that of another siderophore, desferricoprogen, produced from the same fungus, was determined by spectroscopic methods. Triornicin consists of two fragments: dimerumic acid, which is also produced by basic cleavage of desferricoprogen, and N^{α}, N^{δ} -diacetyl- N^{δ} -hydroxyornithine.¹¹¹ There is one report of modest antitumor activity associated with this siderophore, involving a marginal, but reproducible, extension of the mean life span of mice injected with Ehrlich ascites tumor cells (Table 2).¹¹²

Cytochalasin E—Cytochalasin E (Figure 2), belonging to the group of over 60 cytochalasins, was first isolated from *Rosellinia nectarix* and *Helminthosporium dematioideum*.¹¹³ After this, it was isolated from several fungi belonging to different genera, such as *Aspergillus clavatus*,¹¹⁴ *Alternaria chlamydospora* and *Cochliobolus tuberculatus*,¹¹⁵ *Rhinoctadiella* sp.,¹¹⁶ marine-derived fungus *Spicaria elegans*¹¹⁷ and *Aspergillus* sp. Nov F1.¹¹⁸ The most thoroughly investigated biological effects of cytochalasins in cell culture involve the capping of actin filaments, which results in cytokinesis impairment during cell division¹¹⁹ and also affects cancer cell migration properties.¹²⁰ Recently, cytochalasin E also attracted attention due to its antiangiogenic activity. It was found that cytochalasin E was a particularly potent and selective inhibitor of endothelial cells *in vitro* and that it inhibited angiogenesis induced by bFGF and VEGF in mice *in vivo*.¹²¹ These properties led to the evaluation of its effects on tumor growth in the Lewis lung tumor model (Table 2) revealing inhibition of up to 72% at the dose of 2.0 mg/kg administered every other day.¹²¹ Further efforts have been directed toward the development of cytochalasin E analogues lacking the actin activity, which may allow for the administration of the drug at higher doses. In addition, the elucidation of the non-actin target of cytochalasin E will hopefully result in the development of more specific analogues and may reveal new signaling pathways involved in tumor angiogenesis.

Cotylenin A—Cotylenin A (Figure 2), is a diterpenoid carbocyclic closely related to the fusicoccin family of fungal metabolites¹²² in terms of having the same ring system. It was isolated as a plant growth regulator with cytokinin-like activity,^{123,124} together with additional analogues (cotylenins B, C, D and E) from *Cladosporium* sp. and its structure was determined by spectroscopic methods and degradation chemical studies.¹²² Successively, its aglycone, named cotylenol, was isolated from the culture filtrates of a fungus strain 501-7w.¹²⁵ Cotylenin A affected the differentiation of leukemic cells freshly isolated from acute myeloid leukemia (AML) patients in primary culture and stimulated functional and morphologic differentiation.¹²⁶ In an animal study in SCID mice injected with the human promyelocytic leukemia cell line NB4 that resulted in the death of all mice due to leukemia, an administration of cotylenin A significantly prolonged the survival of the mice. It was also shown that cotylenin A induced the differentiation of leukemia cells in a retinoid-resistant leukemia model.¹²⁶ Thus, it appears that cotylenin A may be useful for differentiation therapy of retinoid-resistant leukemia. However, the fact that increasing the dose of cotylenin A in the above-described experiments had little effect because of its low solubility, suggests that the attention should be shifted to the development of more drug-like cotylenin A analogues.

Myriocin—Myriocin (Figure 2), is an antibiotic metabolite produced by *Melanconis flavovirens*, which was found to be identical to the previously isolated thermozycidin.¹²⁷ It was later also isolated from *Isaria sinclairii*, and found to possess potent immunosuppressive activity,¹²⁸ as well as from *Mycelia sterilia*.¹²⁹ Further, it was isolated from *Myriococcum albomyces*,¹³⁰ and as an antifungal metabolite from *Cordyceps heteropoda*, an entomopathogenic fungus isolated from an Australian cicada.¹³¹ The potent immunosuppressive activity associated with myriocin led to the development of its synthetic analogue fingolimod (FTY720) as a United States Food and Drug Administration-approved drug (Gilenya)¹³² for multiple sclerosis. Fingolimod (Figure 2) was found to have a novel mechanism of action. Once ingested, it is rapidly phosphorylated by sphingosine kinase 2 to form fingolimod-P, which resembles the ligand sphingosine 1-phosphate (S1P) and competes with it to bind to four of the five S1P receptors. Because S1P signaling has been shown to increase cell survival, growth, proliferation, intracellular calcium concentration and rearrangement of the actin cytoskeleton,¹³³ fingolimod, together with related synthetic myriocin analogues VPC03090 and OSU-2S (Figure 2), have undergone extensive evaluation as anticancer agents including a number of *in vivo* models (Table 2). All reported studies revealed highly promising results and it appears that cancers such as hepatoma, hepatocellular carcinoma, gastric, prostate and breast tumors, should all be responsive toward S1P receptor antagonists. This in turn should warrant clinical evaluation of these and/or related myriocin analogues. Fingolimod also reduces migration and invasion of human glioblastoma cell lines via inhibition of the PI3K/AKT/mTOR/p70S6K signaling pathway.¹³⁴ This is of importance as it is the process of diffuse GBM cell invasion into the brain parenchyma, which is the major cause of GBM patient death.¹³⁵

Palmarumycin CP1—Palmarumycin CP1 (Figure 2), belongs to the spirobisnaphthalenes, a group of naphthoquinone derivatives with various biological activities including antibacterial, antifungal, antitumoral, among others.¹³⁶ The structure of this metabolite was

determined following its isolation from *Coniothyrium palmarum*, an endophytic fungus isolated from *Lamium purpureum*.¹³⁷ Palmarumycin CP1 was found to be a potent inhibitor of thioredoxin reductase-1, but its evaluation *in vivo* has been hampered by poor water solubility. Thus, its water-soluble prodrug, PX-916 (Figure 2), was synthesized and showed excellent activity in a number of animal tumor models (Table 2), even with cures in some cases.¹³⁸ Thus, a single i.v. dose of PX-916 (25 mg/kg) inhibited MCF-7 human tumor xenograft thioredoxin reductase-1 for at least 48 hours. In addition, the growth of A673 human rhabdomyosarcoma, MCF-7 human breast cancer and SHP-77 small cell lung cancer xenografts was significantly decreased (Table 2). In the latter study, three of eight mice studied, had no histologically detectable tumor when the experiment was terminated on day 42.¹³⁸ These impressive results set the stage for further development of water soluble prodrugs derived from palmarumycin CP1.

Galiellalactone—Galiellalactone (Figure 2), a hexaketide with interesting pharmacological activities, was isolated from four strains of *Galiella rufa* (Sarcosomataceae, Ascomycota) and two unidentified fungi, which by DNA sequence, also appeared to belong to the Sarcosomataceae family. These were a wood-inhabiting apothecial species from Chile and an endophytic isolate from *Cistus salvifolius* (Sardinia).^{139,140} Galiellalactone was also isolated, together with seven related lactones, from the Ascomycete A111-95:2 and its structure elucidated essentially by NMR spectroscopy.¹⁴¹ In 2001, its absolute configuration was established through a total synthesis from (*R*)-(+)-pulegone.¹⁴² Galiellalactone has been found to interfere with the Stat3 signaling pathway and, because the constitutively activated Stat3 has been correlated with the malignant potential of prostate cancer, this natural product was evaluated against prostate cell cultures and in a mouse prostate cancer model (Table 2).¹⁴³⁻¹⁴⁵ Galiellalactone significantly suppressed DU145 and PC-3 xenograft growth *in vivo* and reduced the relative mRNA expression of Bcl-xL and Mcl-1. It was concluded that galiellalactone is a potential anticancer lead against hormone-refractory prostate cancer with constitutively active Stat3.

Epoxyquinol B—Epoxyquinol B (Figure 2), is a pentaketide with a complex, highly oxygenated, heptacyclic structure isolated from an unknown soil fungus and found to have potent antiangiogenic activity.¹⁴⁶ It was isolated together with the closely related epoxyquinols A and C and epoxytwinol A, and the structure of the latter was also confirmed by an asymmetric total synthesis.¹⁴⁷ Later, two other related isoprenylated cytotoxic epoxyquinols, named patsaloquinols A and B, were isolated from *Pestalotiopsis* sp.¹⁴⁸ It was found that epoxyquinol B, containing two electrophilic epoxides, inhibited angiogenesis by covalently binding to the cysteine residues of VEGFR2, EGFR, FGFR, and PDGFR and crosslinking proteins. Thus, it was hypothesized that this pentaketide inhibits signal transduction, including NF-kappaB signaling, through inter- and intramolecular crosslinking of target proteins.¹⁴⁹ In a mouse subcutaneous renal adenocarcinoma model (Table 2), the administration of epoxyquinol B resulted in the reduction of blood vessels supplying the tumor and a decrease in the tumor volume, without significant toxicity.¹⁴⁹ These results therefore warrant further investigation of this promising metabolite as an angiogenesis inhibitor. It must be nevertheless emphasized that angiogenesis inhibitors seem to be

currently less promising for treating cancer patients than what was thought a decade ago because of various types of limiting toxicities for long-term treatments.¹⁵⁰⁻¹⁵⁵

Gliocladicillins A and B—Gliocladicillins A and B (Figure 2), dimeric epipolythiodioxopiperazine alkaloids, were firstly isolated from *Cordyceps*-colonizing fungi using bioguided anticancer fractionation methods.¹⁵⁶ In later studies, gliocladicillin A was isolated together with six related analogues from two filamentous fungi of the Bionectriaceae, strains MSX 64546 and MSX 59553, and its structure was determined by extensive use of NMR and HRMS.¹⁵⁷ Both gliocladicillins A and B displayed significant antiproliferative effects against HeLa cells, inducing a G2/M cell cycle arrest and apoptosis. In addition, they showed significant activity in an *in vivo* subcutaneous B16 mouse melanoma model (Table 2).¹⁵⁶ The results obtained warrant further evaluation of gliocladicillins A and B as anticancer agents.

Apicidin—Apicidin (Figure 2), is a cyclic tetrapeptide produced by some isolates of *Fusarium semitectum*, which exhibits a broad spectrum of *in vivo* antiprotozoal activity against Apicomplexa parasites.¹⁵⁸ Its structure was determined by physical and spectroscopic studies, following its isolation from *Fusarium pallidoroseum*.¹⁵⁹ Later, apicidin was shown to have antiproliferative and cyto-differentiation activity in mammalian cells.¹⁶⁰ Specifically, apicidin inhibits cell proliferation in several human cancer cell lines, including leukemia, cervical cancer, gastric cancer and breast cancer by inhibiting the histone deacetylase enzyme in cancer cells.¹⁶⁰ Promising *in vitro* data prompted its evaluation *in vivo* in mice bearing an Ishikawa endometrial cancer xenograft (Table 2). In these studies, apicidin inhibited cell proliferation and angiogenesis, and induced apoptosis in this endometrial cancer model.¹⁶⁰ The inhibitory effect of apicidin on tumor growth was mediated in part by the up-regulation of acetylated H3 and p21, and the down-regulation of HDAC3 and HDAC4. In another study, the anti-tumor effect of apicidin on SKOV-3 ovarian cancer cells was examined *in vivo* using a carcinomatosis model in nude mice.¹⁶¹ In addition, apicidin significantly inhibited the tumor burden of SKOV-3 cells without noticeable systemic toxicity. Furthermore, apicidin resulted in histone H3 acetylation and repression of HDAC4 expression. These promising results in endometrial and ovarian cancer models warrant its further evaluation against these malignancies.

Chaetocin—Chaetocin (Figure 2), is a diketopiperazine firstly isolated and characterized by chemical and spectroscopic methods, as well as X-ray analysis, from *Chaetomium minutum*.¹⁶² In a successive study, it was isolated from *C. thielavioideum* and *Farrowia* sp.¹⁶³ Recently, it was also isolated as a secondary metabolite from the fungus *C. brasiliense*.¹⁶⁴ Chaetocin was found to exert promising antiproliferative activity in cancer cell cultures, although the precise mechanism is still debated and possibly involves its ability to serve as a competitive substrate (with respect to thioredoxin) and as an inhibitor of the enzyme thioredoxin reductase. Additionally, chaetocin has been found to inhibit histone methyltransferase and HIF-1 α ¹⁶⁵ and also histone lysine methyltransferase SUV39H1 signaling.¹⁶⁶ Its evaluation in SKOV3 ovarian cancer xenografts in nude mice (Table 2) revealed significant tumor growth delay. In addition, the excised tumors were less vascular,

suggesting anti-angiogenic effects.¹⁶⁵ These antiangiogenic effects have recently been demonstrated at the experimental level.¹⁶⁷

Destruxin B—Destruxin B (Figure 2), is a cyclodepsipeptide that was first isolated from *Alternaria brassicae*, the causal agent of gray leaf spot in Brassica plants.¹⁶⁸ It induced chlorosis and necrosis on host and non-host plants, respectively.¹⁶⁹ It was also isolated from *Metarhizium anisopliae*, together with its analogue destruxin A, and showed insecticidal activity.¹⁷⁰ Later, destruxin B was also isolated together with additional analogues from the fungus *Cordyceps indigotica*.¹⁷¹ Studies have shown that destruxins perturbed the syntheses of DNA, RNA and proteins and that the compound also has antiviral activity.¹⁷² The promising anticancer effects were revealed by the study of destruxin B in colorectal cancer models and specifically as an inhibitor of the Wnt/beta-catenin/Tcf signaling pathway in colorectal cancer (Table 2)^{172,173} and hepatocellular carcinoma¹⁷⁴ cells, with promising results. In addition, promising anticancer activity has also been recently demonstrated for destruxin B in oral cancer cells.¹⁷⁵

3.2. In Summary

3.2.1. Fungal Metabolites that Are Unlikely to Move to Clinical Trials—It was shown in the early 1980s that triornicin (Figure 2) displayed weak *in vivo* antitumor activity (Table 2). However, no patents have been filed for triornicin or its analogues and clinical trials are unlikely in the immediate future. Cytochalasin E belongs to a class of actin-binding molecules with various levels of cardiotoxicity.¹¹⁹ More than 30 patents have been filed since the 1970s for cytochalasin E. However, only two of them are related to antitumor activity and they were filed in the early 1990s^{181,182} without any further interest in their development. While galiellalactone, and its derivatives (Figure 2), display promising anticancer activity in prostate cancer preclinical models (Table 2), again no patents have been filed as of today with respect to their use as potential anticancer drugs. Finally, epoxyquinol B and its analogues display antiangiogenic activity, which does not appear to be a highly promising way to treat cancer patients as explained above. In addition, no patent has been filed with respect to their anticancer activity.

3.2.2. Fungal Metabolites that Could Move to Clinical Trials—Cotylenin A (Figure 2) could be useful for differentiation therapy of retinoid-resistant leukemia (Table 2). A first patent was filed in 1992 with respect to the antitumor effects of cotylenin analogues,¹⁸³ and then a second one in 2008.¹⁸⁴

Myriocin and several of its analogues (Figure 2) are antagonists of S1P signalling, which is involved in cell proliferation increase (cell growth kinetics) and actin cytoskeleton rearrangements (cell migration kinetics) (Table 2). Myriocin synthetic analogues such as VPC03090 and OSU-2S (Figure 2) have undergone extensive evaluation as anticancer agents, in numerous *in vivo* tumor models with very promising results (Table 2). About 30 patents have been filed between 1975 and 2013 for myriocin. In addition, several patents have been filed for the immunosuppressive drug FTY720 (Gilenya), a synthetic analogue of myriocin (Figure 2), for diseases such as inflammatory demyelinating diseases¹⁸⁵ and type 2

diabetes.¹⁸⁶ In contrast, no patent has yet been applied for FTY-720 or its nonimmunosuppressive analogue OSU-2S in a specific cancer domain.

Palmarumycin and/or its analogues (Figure 2) seem very promising in treating various cancer types, at least at the preclinical level (Table 2). Five patents relating to the antitumor effects of palmarumycin and/or its analogues have been filed between 2002 and 2010.¹⁸⁷⁻¹⁹¹

For the moment no patent has been filed for the gliocladicillins as potential anticancer drugs. The possibility remains that patents will be filed in the future because only two recent publications report the anticancer activity of this set of compounds.^{156,157}

Apicidin is a HDAC inhibitor and 3 patents have already been filed with respect to its anticancer activity.¹⁹²⁻¹⁹⁴

Chaetocin displays anticancer activity through several mechanisms as detailed above. Three patents have already been applied for chaetocin as a potential anticancer agent.¹⁹⁵⁻¹⁹⁷

Destruxin displays interesting anticancer properties, including for example modulation of the Wnt/beta-catenin pathway (see above). Four patents have been filed for destruxin, but not in the cancer field (e.g., for the control of insects,¹⁹⁸ as a cardiotonic agent¹⁹⁹ and for treating osteoporosis).^{200,201}

4. Conclusion

In terms of finding new ways of treating cancer in humans, methods based on small molecule chemotherapeutics continue to be of significant importance. Natural products have long been a valuable source of the molecular entities, making it particularly important that the environments producing these compounds are protected. In this review, the potential of fungal metabolites to provide a marketed cancer drug is discussed. Examples of metabolites, and their derivatives or analogues, which have been evaluated in oncology-related clinical trials, include anguidine, aphidicoline, rhizozin, fumagillin, illudin S, phenylahistin and wortmannin. Quite surprisingly, although a few of the compounds were tested at Phase II level, none of these compounds have advanced further. In our opinion, plinabulin and PX-866, halimide and wortmannin analogues respectively have the most potential if ongoing trials are successful.

It was also noted that a significant number of fungal metabolites have been successfully tested in a wide variety of mouse cancer models. These include triornicin, cytochalasin E, cotylenin A, myriocin, palmarumycin CP1, galiellalactone, epoxyquinol B, gliocladicillins A and B, apicidin, chaetocin and destruxin B. Also of importance is that recent patents, for the use of the metabolites and/or their analogues/derivatives in oncology, have been filed for cotylenin A, myriocin, palmarumycin CP1, apicidin and chaetocin and there is thus the possibility that these compounds will advance to clinical trials.

With the fungus kingdom being remarkably diverse, in terms of species and the structures (metabolites) produced by them, it is thus surprising that only a few handfuls of these compounds have made it to clinical trials, particularly as the need for new cancer

chemotherapeutics is so important. Furthermore, estimations that only a small percentage of fungi have been investigated, along with technical challenges associated with the cultivation and biochemical-guided isolation of metabolites, means that this area of natural product research still has much to offer the field of anti-cancer research. In addition, the development and application of new strategies to induce the fungi to expand the structural diversity of the metabolites produced, could lead to these organisms being seen as Nature's own "bio-combinatorial reactors". The most significant advantage of this approach would be that each of the metabolites developed in this manner would also be "privileged" due to their biochemical origin.

In summary, despite there being no clinical anti-cancer agents based on fungal metabolites currently being used for the treatment of patients with cancer, it appears to only be a matter of time before compounds from this class of natural products will be added to the anti-cancer pharmaceutical armamentarium.

Acknowledgments

RK is a director of research with the Fonds National de la Recherche Scientifique (FRS-FNRS, Belgium). AK thanks the Welch Foundation (Grant No. AI-0045), National Institutes of Health (1R15-CA186046-01A1) and National Science Foundation (grant 0946998) for financial support. WvO acknowledges the National Research Foundation (South Africa) and Stellenbosch University for funding.

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Figure 1. Fungal metabolites and/or their analogues that have entered human cancer clinical trials

Figure 2. Fungal metabolites and/or their analogues tested in in vivo models of human cancer

Table 1

Compound Name	Biological Target	Trial Design	Study Population	Outcome	References
Anguidine	Protein synthesis inhibition	Broad Phase II study of solid cancers	276 Patients with gastric cancer, prostatic cancer, adenocarcinoma of the stomach, renal cancer, adenocarcinoma of the lung	No meaningful activity of anguidine in six tumor categories was found. Substantial, but not prohibitive, myelotoxicity and acceptable nonmyelosuppressive toxic effects were observed.	59-64
Aphidicolin glycinate – synthetic analogue of aphidicolin	Inhibition of DNA polymerase alpha and delta	Two consecutive Phase I clinical studies to evaluate toxicity profile and pharmacokinetics	24 Healthy volunteers	Dosage and administration schedule was identified for clinical evaluations of aphidicolin glycinate as a single agent or in combination with cisplatin	69
Rhizoxin	Vinca site of tubulin, inhibition of microtubule assembly	Phase I study to test the feasibility of a 72-hour continuous i.v. infusion	19 Patients with solid cancers	Rhizoxin can be safely administered using a 72-hour continuous i.v. infusion schedule	73
		Phase II study to determine clinical efficacy	26 Melanoma patients	Patients were tolerant to the dosage administered (2 mg/m ² every 3 weeks), but no significant improvement in patient conditions were observed.	74
		Phase II study to determine clinical efficacy	32 Patients with recurrent and/or metastatic squamous cell head and neck cancer	The used dose and schedule (1.5-2.0 mg/m ² i.v. bolus injection once every 3 weeks) led to only minor activity	75
		Multicenter Phase II study to determine clinical efficacy	31 Chemotherapy-naïve patients with advanced NSCLC	The median duration of response was 7 months and median survival from the start of rhizoxin treatment was 6 months. Rhizoxin as a single agent shows activity in patients with advanced NSCLC.	76
TNP-470 - fumagillin analog	Methionine aminopeptidase 2, inhibition of angiogenesis	TNP-470 was administered i.v. over 4 hours weekly	36 Patients with advanced cancer including melanoma (4), NSCLC (3) and squamous cell head and neck cancer (2)	TNP-470 was well-tolerated and one patient with malignant melanoma showed stabilization of the disease progression.	99, 100
		Phase I dose escalation trial of alternate-day i.v. TNP-470	33 Patients with metastatic and androgen-independent prostate cancer	No definite antitumor activity of TNP-470 was observed	101
		Phase I study to investigate the synergistic application of TNP-470 with paclitaxel	16 Patients diagnosed with therapeutically difficult to treat NSCLC	6 (38%) Patients showed partial responses. The combination was generally well tolerated by the patients, and due to the impressive survival rate, the TNP-470/paclitaxel cocktail should receive more investigation.	102
		Toxicity and pharmacokinetics of carboplatin in combination with TNP-470 and paclitaxel	17 Patients with lung, head/neck cancer, sarcoma and thymoma	Combination of TNP-470, paclitaxel, and carboplatin is a reasonably well tolerated regimen	103
PPI-2458 - fumagillin analog	Methionine aminopeptidase 2, inhibition of angiogenesis	A Phase I dose escalation safety/tolerance study	Patients with non-Hodgkin's lymphoma or solid tumors who failed prior treatments or were refractory to standard therapy	Oral QOD for 28-day cycles was found to be safe and well tolerated.	82, 104

Compound Name	Biological Target	Trial Design	Study Population	Outcome	References
CKD-732 - fumagillin analog	Methionine aminopeptidase 2, inhibition of angiogenesis	Phase I trial of the antiangiogenic agent CKD-732 to determine the maximum tolerated dose (MTD), pharmacokinetics (PK), and safety profiles	19 Patients with refractory solid tumors	Confusion and insomnia were dose-limiting toxicities (DLTs), and MTD was 15 mg/m ²	105
		A Phase Ib pharmacokinetic study of CKD-732 in combination with capecitabine and oxaliplatin	Metastatic colorectal cancer patients who progressed on irinotecan-based chemotherapy	The Phase II recommended dose of CKD-732 was determined to be 5 mg/m ² /d	106
Irofulven - illudin S analog	DNA-targeting alkylating agent	A trial to determine the safety and efficacy as a single agent	Patients with recurrent ovarian cancer who had received extensive prior chemotherapy	Dosing at 0.55 mg/kg had persistent retinal toxicity, yet demonstrated only limited antitumor activity	87
		Study to determine the MTD, recommended dose, dose limiting toxicities, safety and pharmacokinetics of irofulven combined with capecitabine	Advanced solid tumor patients	12 Patients had disease stabilization > 3 months	107
		Study to evaluate the tolerability and efficacy of irofulven as a single agent	23 Patients with recurrent or metastatic gastric cancer	Irofulven was tolerated at the dose of 0.45 mg/kg on days 1 and 8, every 3 weeks but showed no evidence of antitumor activity.	108
		Multicenter Phase II trial to evaluate the activity and the safety of irofulven as a single agent	61 Patients with recurrent epithelial ovarian cancer	There were 7 partial responses (12.7%), and 30 patients (54.6%) had stable disease.	109
NPI-2358 (plinabulin) - synthetic analogue of halimide	Colchicine binding site on beta-tubulin, vascular disrupting agent	Phase I study to assess the recommended Phase 2 dose of plinabulin combined with docetaxel	13 Patients including 8 with NSCLC	Among patients with NSCLC 2 achieved a partial response and 4 demonstrated decreases in tumor measurements	89
PX-866 - synthetic analogue of wortmannin	PI3Ks and PIKKs inhibition	Phase I study to evaluate for MTD, safety, pharmacodynamics, pharmacokinetics, and antitumor activity	84 Patients with incurable cancers	Treatment was well tolerated and was associated with prolonged stable disease	110

Table 2

Compound	Biological Target	Mouse Model	Results	Reference
Triornicin	Iron transport	30 g Swiss Tex mice with i.p. injection of Ehrlich ascites tumor cells	Daily i.p. injections of triornicin (0.1 mL in aqueous solution at a range of concentrations from 0.02 to 0.002 mg/mL) for a total of 14 days provided a modest increase in lifespan from 10% to 36% vs. the control group.	112
Cytochalasin E	Angiogenesis inhibition	Lewis lung tumor in mice	At 1.0 mg/kg/day, cytochalasin E led to 23% tumor growth inhibition. A dose of 2.0 mg/kg every 3 days gave approximately the same degree of inhibition. Increasing the dose to 2.0 mg/kg administered every other day resulted in 72% inhibition. Higher doses resulted in weight loss. The results showed that cytochalasin E was an effective inhibitor of tumor growth, as well as angiogenesis.	121
Cotylenin A	Possibly a member of the family of 14-3-3 proteins	SCID mice that had been inoculated with human promyelocytic leukemia NB4 cells	Cotylenin A significantly prolonged the mean survival time of mice inoculated with NB4 cells. The mean survival time of mice inoculated with NB4 cells was 32 days, and this increased to 45 days when mice were treated with cotylenin A.	126
Fingolimod (FTY720) – synthetic analogue of myriocin	Sphingosine 1-phosphate (S1P) antagonism	CWR22R - an androgen-independent human prostate tumor xenograft inoculated into castrated nude mice	An i.p. injection of FTY720 at 10 mg/kg for 20 days led to suppression of CWR22R tumor growth, without causing any detectable side effects in nude mice. The FTY720-induced tumor suppression was correlated with decreased serum PSA level. The results suggest FTY720 to be a promising agent in the suppression of androgen-independent prostate cancer.	176
		Rat orthotopic liver tumor model established by injection of a buffalo hepatoma cell line MH7777 into the right portal vein.	An i.p. injection of FTY720 at 5 mg/kg/day markedly reduced the proliferation index of tumor cells to 15% compared with that of 43% in the control group ($p < 0.001$). It was concluded that FTY720 is an effective anticancer agent for liver tumor in a rat model.	177
		Human gastric cancer cell xenografts in nude mice established with MGC803 cell line	An i.p. injection of FTY720 at 10 mg/kg/day for 20 days led to significantly suppressed tumor growth in the FTY720-treated mice compared with control, while the body weight of mice from the treated group was similar to the control group. These data imply that FTY720 is a potential therapeutic treatment of gastric cancers and that it is relatively non-toxic to nude mice.	178

Compound	Biological Target	Mouse Model	Results	Reference
		Assessment of a simultaneous blockade of the PDGF and S1P pathways on the chemotactic responses of VSMCs and its effects on breast tumor growth by using a combination of sunitinib malate and fingolimod in a rat breast tumor growth in a syngeneic cancer model (Walker 256)	This orally administered bi-therapy resulted in normalization of the tumor vasculature without cumulative toxicity. The simultaneous blockade of PDGF and S1P pathways with sunitinib malate and fingolimod may provide an effective means of reducing tumor angiogenesis, and may improve the delivery of other chemotherapies.	179
VPC03090 - synthetic analogue of myriocin	Sphingosine 1-phosphate (S1P) antagonism	4T1 mammary carcinoma in mice	An i.p. injection of VPC03090 at 6.2 mg/kg/day reduced the median tumor volume 3-fold compared with vehicle treatment. This finding warrants further investigation using VPC03090 and other drug-like S1P antagonists in additional cancer models.	133
OSU-2S - synthetic analogue of myriocin	Sphingosine 1-phosphate (S1P) antagonism	Assessment of efficacy of OSU-2S <i>vis-a-vis</i> FTY720 in both ectopic and orthotopic Hep3B hepatocellular carcinoma tumor xenograft models.	An i.p. injection once daily with OSU-2S or FTY720 at 5 and 10 mg/kg, or with vehicle. Both agents, at 5 mg/kg, completely suppressed Hep3B tumor growth relative to the vehicle control ($P < 0.001$). The findings suggest that OSU-2S has clinical value for HCC and warrants its further investigation.	180
PX-916 - Palmarumycin CP1 prodrug	Thioredoxin reductase-1	A673 human rhabdomyosarcoma xenografts	Tumor growth was significantly decreased at 30 mg/kg/d, i.p., after 5 doses.	138
		SHP-77 small cell lung cancer	Tumor growth was significantly decreased at 25 mg/kg/d, i.v., after 5 doses. 3/8 mice had no detectable tumor when examined on day 42.	138
		MCF-7 human breast cancer xenografts	Tumor growth was significantly decreased at 22.5 mg/kg/d, i.v., after 5 doses (52% inhibition).	138
Galiellalactone	Stat3 signalling	DU145 and PC-3 subcutaneous xenografts in mice	Daily i.p. injections of galiellalactone at 1 mg/kg significantly reduced the tumor growth rate in DU145 xenografts by 41–42% compared to control mice treated with vehicle (0.1% ethanol). PC-3 xenograft growth was reduced by 25% compared to the control group. Thus, it was concluded that galiellalactone is a potential anti-tumor lead against hormone-refractory PCa with constitutively active Stat3.	143, 144
Epoxyquinol B	VEGFR2, EGFR, FGFR, and PDGFR, angiogenesis inhibition	Balb/c mouse subcutaneous xenograft of Renca (mouse renal adenocarcinoma) cells	An i.p. administration of epoxyquinol B at 3 or 10 mg/kg every other day resulted in the reduction of blood vessels supplying the tumor and the tumor volume without significant toxicity. The results showed that epoxyquinol B was an effective inhibitor of tumor growth, as well as angiogenesis.	149
Gliocladicillins A and B	Unknown	C57BL/6J mice injected subcutaneously with B16 melanoma cells	Injections with gliocladicillin A (0.25 and 0.50 mg/kg) or gliocladicillin B (0.10 and 0.40 mg/kg) once a day for 21 days. showed significant anti-tumour efficacy with inhibition levels of 69.8–87.2% for gliocladicillin A and 56.7–82.5% for gliocladicillin B.	156

Compound	Biological Target	Mouse Model	Results	Reference
Apicidin	Histone deacetylase inhibition	Balb/c nude mice bearing an Ishikawa endometrial cancer xenograft	Significant inhibition of tumor growth was observed starting from day 15 after the apicidin treatment (5 mg/kg) up to 53% relative to the control group.	160
		Carcinomatosis SKOV-3 ovarian model in nude mice	An i.p. treatment with (5 mg/kg) or with vehicle every other day over a 24 day period showed that after 26 days the apicidin-treated mice had significantly reduced tumor burden by 69% relative to the vehicle-treated controls.	161
Chaetocin	Possible targets: thioredoxin reductase, histone methyltransferase and/or HIF-1a signaling	SKOV3 ovarian cancer xenografts in nude mice	An i.p. injection at 0.2 mg/kg, 5 times per week, significantly delayed the growth of established SKOV3 tumors with minimal evidence of toxicities observed in treated animals.	165
Destruxin B	Inhibition of Wnt/beta-catenin/Tcf signaling pathway	Murine xenograft model of human HT-29 colorectal cancer	Subcutaneous administration of destruxin B daily at 0.6-15 mg/kg was proven to be safe and effective in inhibiting the growth of colorectal cancer cells. The increase in tumor volumes of treated groups were significantly ($p<0.05$) lower than those of the mock-treated group.	172
		Study of tumorigenesis in HT29 xenograft mice using non-invasive bioluminescence technique	Destruxin B inhibited tumorigenesis in HT29 xenograft mice using a non-invasive bioluminescence technique. Suppressed expression of beta-catenin, cyclin D1, survivin, and endothelial marker CD31 was noted, while caspase-3 expression increased.	173