ChemInform Abstract: Anticancer Compounds Derived from Fungal Endophytes: Their Importance and Future Challenges

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REVIEW

Anticancer compounds derived from fungal endophytes: their importance and future challenges

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Covering: 1990 to 2010

This is a review of anticancer agents isolated from endophytic fungi from 1990–2010. Endophytic fungi are defined as fungi that live asymptomatically within the tissues of higher plants. The designation 'anticancer' is based on the assessment of the authors of the paper of the cytotoxicity of each compound against specific cancer cell lines. Many of the compounds reported here were isolated exclusively from endophytes in culture, while other compounds had been previously reported as chemical constituents of higher plants. The uniqueness of the endophytic community of fungi is stressed as a promising source of novel compounds with anticancer activity, or as an alternative source of compounds originally isolated from higher plants. Endophytes represent a dependable source of specific secondary metabolites, and can be manipulated both physicochemically and genetically to increase yields of desired metabolites and to produce novel analogues of active metabolites.

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1 Introduction

1.1 Natural products as a source of medicinal compounds

Cancer is a group of diseases that can affect various organs of the body, and is characterized by the uncontrolled growth of abnormal cells and invasion into normal tissue. Cancer cells can also spread to other parts of the body and produce new tumors. If the spread of cells becomes uncontrolled, it can lead to death. In 2007, it was estimated that cancer killed 7.6 million people around the world. The annual death toll from cancer is expected to rise to 17.5 million by 2050, simply due to projected population growth and aging, as well as lifestyle and environmental influences, including smoking and exposure to carcinogenic agents.[1] Access to a limited number of cancer chemotherapies,

their deleterious side effects and high cost of most (if not all) of these drugs, make disease treatment especially difficult. Furthermore, many existing therapies do not effectively treat certain cancers, and multi-drug-resistant tumors exacerbate treatment challenges. The discovery of new chemotherapeutic agents is a key goal for natural product and medicinal chemists.

Secondary metabolites (natural products) have played an important role in the discovery and development of medicinal agents. In their analysis of data reported in *Annual Reports of Medicinal Chemistry*, Newman *et al.* found that from 1989–1995, over 60% of the approved drugs and drug candidates developed as anti-infective agents or anticancer drugs were of natural origin.[2] These excluded biologics (peptides or proteins >45 residues isolated from organisms or cell lines, or produced in a surrogate host)

Ravindra Kharwar

Ravindra Kharwar carried out his B.Sc. (1987), M.Sc. (1989) and Ph.D. (1997) at Gorakhpur University, Gorakhpur. During his Ph.D., he worked on fungal taxonomy, and then he joined the Centre of Advanced Study in Botany, BHU, Varanasi, as an Assistant Professor, in February 1997. Since then, he has worked on various aspects of fungal endophytes, including a period at Montana State University, USA, with Professor Gary Strobel in 2007–2008. In 2009 he was promoted to Associate Professor, and recently has been working on ecology, biodiversity, bioactive molecules and biosynthesis of metal nanoparticles using endophytic fungi and actinomycetes.

Ashish Mishra

Ashish Mishra received his B.Sc. (2002) and M.Sc. (2005) degrees from VBS Purvanchal University, Jaunpur. Since 2007, he has been carrying out his Ph.D. research under the guidance of R. N. Kharwar, in the Department of Botany, on the topic 'Assessment of endophytic mycoflora of some medicinal plants for potential antimicrobial substances'. He has studied some medicinal plants of the eastern parts of Uttar Pradesh, India, and has isolated considerable numbers of endophytic fungi. Currently, he is involved in screening of those fungi to isolate bioactive natural products.

Surendra Gond

Surendra Gond graduated in 2002 and received his post-graduate degree in 2004 from BHU, Varanasi. He passed the National Eligibility Test, Junior Research Fellow, in 2004 organized by CSIR, New Delhi. Since 2005, he has also been carrying out a Ph.D. degree under the supervision of R. N. Kharwar, and has been involved in the isolation and characterization of bioactive natural products and the biofabrication of metal nanoparticles using endophytic fungi.

Andrea Stierle

Andrea Stierle earned a doctorate in Chemistry from Montana State University, where she discovered the first host-specific toxin against the weed pest spotted knapweed. With husband and collaborator Donald Stierle she has studied sponge endosymbionts as sources of new bioactive agents, and discovered a unique antibiotic that was also active against the AIDS virus. While at Montana State University, she (in collaboration with her husband) discovered that a fungus in the bark of the Pacific yew tree, Taxus brevifolia, *produced taxol in* de novo *fashion, and this fungus –* Taxomyces andreanae *– bears her name. As a Research Professor at the University of Montana, she and Donald continue to work with microbes isolated from unusual ecological niches. She is currently studying microbes from an acid mine waste lake as sources of novel bioactive metabolites.*

Donald Stierle

Donald Stierle earned his doctorate in Chemistry from the University of California Riverside. He was a Professor of Chemistry and held the Rose and Ann Busch Endowed Chair at Montana Tech. He started as a marine natural products chemist, and with his wife Andrea studied the metabolites of sponge endosymbionts, discovering a new antibiotic with activity against the AIDS virus. He collaborated with his wife on the discovery of a taxol-producing fungus in the bark of the Pacific yew tree, and they explored other endophytes from yew trees and redwoods for new bioactive metabolites. As a Research Professor at the University of Montana, he continues to work with microbes isolated from unusual ecological niches. He is currently studying microbes from acid mine waste lake as sources of novel bioactive metabolites.

but included original natural products, derivatives of natural products, and synthetic products modeled on natural compounds.[2]

In a detailed analysis of new medicinal agents over the period of 1981–2002, Newman *et al.* carefully deconstructed the compound sources of new and approved drugs for diseases ranging from analgesics to vulneraries.[3] Of particular interest are the new anticancer agents approved during this period. Of the 79 new anticancer agents, 12 are biologics, 9 are natural products, 21 are natural product derivatives, 10 were derived from total synthesis with a natural product pharmacophore, and 25 are from total synthesis. They included a new category for the synthetic molecules – natural product mimics. Approximately 30% of the synthetic compounds (including those with natural product pharmacophores) were "designed from knowledge gained from the natural product" or "discovered by using an assay whereby the compound is designed to displace the natural substrate in a competitive fashion".[3]

1.2 Plant-derived natural products as a source of anticancer agents

Many important anticancer drugs have been isolated from plant sources. These compounds include the vinca alkaloids, vinblastine and vincristine, which were isolated from the Madagascar periwinkle, *Catharanthus roseus*,[4–6] and paclitaxel (Taxol®).[7] The leaves of *C. roseus* have long been used to treat a variety of diseases, and researchers have reported hypotensive, hypoglycemic and purgative properties for the plant.[8] The antitumor properties of the plant were discovered independently by two teams in the 1950s. While looking for a treatment for diabetes, Noble and Beer found that leaf extracts of *C. roseus* had a strong effect on bone marrow and white blood cells. They ultimately isolated the active principle, a compound which they named vincaleukoblastine, which was later changed to vinblastine.[4] Investigators at Lilly laboratories also isolated vinblastine as well as a new alkaloid, vincristine.[5,6] Semisynthetic derivatives of the vinca alkaloids led to the development of vinorelbine, a more lipophilic drug than the natural products, which is currently used for the treatment of non-small-cell lung cancer.

Paclitaxel was originally isolated from the inner bark of the Northwest Pacific yew tree, *Taxus brevifolia*.[7] In their original report of the structure of paclitaxel, Wani and Wall noted its cytotoxicity against L-1210, P-388, and P-1534 leukemias, its activity as an inhibitor of WM-256 carcinosarcoma, and its considerable cytotoxicity in the 9KB (nasopharyngeal cancer) assay.[7] Its mode of action as a stabilizer of tubulin polymerization is unique in the world of anticancer drugs.[9] Taxol® (paclitaxel) is used primarily in combination with other cancer drugs for the treatment of a variety of cancers, including ovarian, breast, leukemias, lymphomas, advanced testicular, lung cancers and Kaposi's sarcoma.[9] There have been numerous attempts to derivatize paclitaxel to enhance its bioavailability and to reduce its toxic side-effects. One of the most important of these derivatives is docetaxel, a drug with better solubility characteristics than the parent compound.[9]

Unfortunately, there are problems associated with the use of plant-derived natural products, one being that potent cytotoxic metabolites are often produced in very low quantities by the source organisms. For instance, paclitaxel constitutes only 0.01–0.03% of the dry phloem weight of *Taxus*.[9] Supply issues may also be a serious concern if a source plant is endangered or has been collected in a politically quixotic part of the world. There may be seasonal, geographic, or environmental variations in secondary metabolite composition, making the reisolation of a desired compound problematic. Many scientists have looked to microorganisms as a source of new compounds to combat the complex of diseases called cancer.

1.3 The microbial advantage in natural product drug discovery

There are many reasons for studying the secondary metabolites of microorganisms for biological activity. First, bacteria and fungi have existed for over a billion years. During this time they have evolved biosynthetic pathways and mechanisms for synthesizing a rich arsenal of complex secondary metabolites. Most of these compounds interact with enzyme targets and help the organism survive against a wide array of challenges. Each new microbe has the potential for yielding as-yet undiscovered compounds with bioactivity that can be adapted for medicinal purposes. It has been estimated by Demain and others that fewer than 16% of the fungal species that have been described have been cultured and studied. These described species probably represent fewer than 5% of the total fungal species that await exploration.[10]

There are several practical advantages to a microbial source of a desired natural product. Many microorganisms can be stored indefinitely, ensuring availability of the source organism in perpetuity.[11] Microbes can be grown in large-volume tank fermentors, producing a virtually inexhaustible supply of a desired metabolite.[11] Microorganisms typically respond favorably to routine culture techniques, and productivity amplification is relatively easy in microorganisms. In the case of penicillin, improved culture conditions and genetic manipulation of producing strains of *Penicillium* increased drug yield from a few micrograms per millilitre to thousands of micrograms per millilitre.[11] Different bioactive compounds can be produced by altering culture conditions. Directed changes in culture conditions can be explored indefinitely as a method of optimizing various biosynthetic pathways, and result in the production of diverse derivatives of a lead compound.[11] Finally, gene insertion and other molecular techniques are relatively straightforward in microbes, and can be used to up-regulate production of a specific compound or to generate analogues of a lead compound.

2 Endophytic fungi

2.1 Plant endophytes are an under-studied niche in microbial drug discovery

Investigating the secondary metabolites of microorganisms from unusual or specialized niches may increase the chances of finding novel compounds. Scientists often focus their efforts on fungi that cause problems either as animal or plant pathogens. Plant endophytes are more subtle, rarely causing problems, coexisting with their hosts under most circumstances. They are generally nonpathogenic in nature, but may produce secondary metabolites that enable them to survive in the competitive world of plant interstitial space. An overview of recent literature indicated that 51% of bioactive substances isolated from endophytic fungi were previously unknown, compared to 38% from soil fungi. Since the original discovery of a fungus that produced paclitaxel in a *de novo* fashion,[12] approximately 100 compounds with demonstrated anticancer activity have been isolated from endophytic

fungi – including several compounds originally found in other higher plants. This review will describe each of these compounds in terms of their biological activity, the source microorganism and the plant host.

2.2 The definition of an endophyte

The term 'endophyte' was introduced by De Bary[13] in 1866 to define all microbes (including fungi, bacteria, cyanobacteria, and actinomycetes) that reside within plant tissue. Various investigators have defined endophytes in different ways, but Bacon and White have provided a conclusive and widely accepted definition of endophytes: "microbes that colonize living, internal tissues of plants without causing any immediate, overt negative effect".[14] Endophytes include both fungi and bacteria,[15] although there are more published accounts of fungal endosymbionts. The most studied fungal endophytes belong to the ascomycetous family Clavicipitaceae, and colonize temperate-zone grasses.[16] There is evidence that endophytes enhance host resistance against herbivores,[17] insects,[18] disease,[19] reduced seed production,[20] drought,[21] plant pathogens,[22] and against variations in temperature and salinity.[23] Several endophyte-derived natural products have shown potential as antifungal agents against a variety of plant and human pathogens.[24] However, the focus of this report are anticancer agents produced by fungal endophytes.

2.3 Biodiversity and distribution of fungal endophytes

Fungal endophytes are a diverse and versatile group of microorganisms that colonize plants in the Arctic[25] and Antarctic,[26] and in geothermal soils,[23] deserts,[16] oceans,[27] rainforests,[28] mangrove swamps,[29] and coastal forests.[30] They have been isolated from the root complexes and aerial parts of a diverse range of hosts including algae,[27,31] bryophytes,[32] pteridophytes,[33] gymnosperms,[34] and angiosperms.[35,36] Endophytes have been found in every plant studied to date. There are over 300,000 higher plant species, and it can be assumed that each of these species hosts a complex community of endophytic microbes.[37] Endophytes can infect virtually 100% of a host population or a small fraction of the population.[15,38] Although the relationship between endophytes and their hosts varies from organism to organism, fungal endophytes are an important component of microbial biodiversity. These subtle inhabitants of the tissues of higher plants may represent a rich source of as-yet undiscovered genera to contribute to fungal diversity.[37]

3 Cytotoxic natural products from fungal endophytes

3.1 Cytotoxicity and anticancer activity

The first chemotherapeutic agent was discovered quite by accident over fifty years ago. Mustard gas (1,5-dichloro-3-thiapentane) was used as a chemical warfare agent during World War I and was studied further during World War II. During a military operation in World War II, a group of people were accidentally exposed to mustard gas, and were later found to have very low white blood cell counts.[39] Scientists reasoned that an agent that damaged the rapidly growing white blood cells might have a similar effect on certain cancers of the blood. In the 1940s, several patients with advanced lymphomas were given the drug by vein, rather than by breathing the irritating gas. Their improvement, although temporary, was remarkable.[39] This experience led researchers to look for other substances that might have similar effects against cancer.

Many of the anticancer chemotherapeutics widely prescribed today – including antimetabolites, tubulin inhibitors, alkylating agents, and compounds that target DNA-topoisomerases I and II – are cytotoxic (cell-killing) agents. These compounds are designed to kill cancer cells more effectively than normal cells because they generally target the more rapidly dividing cancer cells. However, this is not always the case. Bone marrow cells, hair follicles and epithelial cells such as those lining the GI tract also divide rapidly and are often the targets of side effects that can range from unpleasant to seriously debilitating. Despite the problems associated with the use of cytotoxic agents, cytotoxicity assays using a wide array of cancer cell types have played an important role in the discovery of compounds like paclitaxel, camptothecin and the vinca alkaloids that target cancer cells.[40,41] In this report anticancer activity is generally associated with the cytotoxicity of the compounds described.

3.2 Contribution of endophytic fungi to the discovery of anticancer agents

Endophytic fungi are the hidden members of the microbial world, and because they generally exist asymptomatically, they have received less attention than their more pathogenic relatives. Thus they represent an under-utilized resource in the search for new compounds from unexplored microbes. Studies of these organisms indicate that they are prolific producers of compounds that can be exploited as both agrochemical and medicinal agents. The search for new compounds is certainly important. Of equal importance, however, has been the discovery that some endophytes produce compounds that have been exclusively isolated from higher plants.[12] Following the initial report of the production of paclitaxel from a Northwest Pacific yew endophyte in 1993,[12] researchers have reported the isolation of several other important anticancer agents from fungal endophytes including camptothecin and several analogues,[42–44] vincristine,[45–47] and podophyllotoxin.[48,49]

One hundred anticancer compounds belonging to 19 different chemical classes with activity against 45 different cell lines have been isolated from over 50 different fungal species belonging to 6 different endophytic fungal groups (Table 1). Of the total compounds isolated from endophytic fungi, 57% were novel or were analogues of known compounds. There has been a significant increase in the number of anticancer compounds isolated from endophytic fungi following the first report of the production of paclitaxel by a fungus.[12] There are many different ways to categorize compounds in a review. In this report, compounds will be listed by chemical classification, although some compounds could be assigned to multiple chemical classes.

3.3 Secondary metabolites of endophytic fungi

3.3.1 Aldehydes. Chaetopyranin **1** is a benzaldehyde derivative isolated from the endophytic fungus *Chaetomium globosum* associated with the marine red alga *Polysiphonia urceolata*.[27] Chaetopyranin **1** exhibited moderate or weak cytotoxic activities against three human tumor cell lines: HMEC (human

Table 1 Anticancer compounds isolated from endophytic fungi[a]

Host	Fungal endophyte	Compound	Chemical nature	Cell line/Target enzyme	Activity	Ref.
Polysiphonia urceolata	*Chaetomium globosum*	Chaetopyranin **1**[a]	Aldehyde	HMEC	15.4 μg/mL[b]	27
				SMMC-7721	28.5 μg/mL[b]	27
				A549	39.1 μg/mL[b]	27
Nothapodytes foetida	*Entrophospora infrequens*	Camptothecin **2**	Alkaloid	A549	—	42
				HEP-2	—	42
Camptotheca acuminata	*Neurospora crassa*	Camptothecin **2**	Alkaloid	—	—	43
Camptotheca acuminata	*Fusarium solani*	Camptothecin **2**	Alkaloid	OVCAR-5	—	44
		9-Methoxycamptothecin **3**	Alkaloid	—	—	44
		10-Hydroxycamptothecin **4**	Alkaloid	—	—	44
Catharanthus roseus	*Fusarium oxysporum*	Vincristine **5**	Alkaloid	—	—	45,47
Imperata cylindrica	*Chaetomium globosum*	Chaetoglobosin U **6**[a]	Alkaloid	KB cell line	16.0 μM[b]	58
		Chaetoglobosin C **7**	Alkaloid	KB cell line	34.0 μM[b]	58
		Chaetoglobosin F **8**	Alkaloid	KB cell line	52.0 μM[b]	58
		Chaetoglobosin E **9**	Alkaloid	KB cell line	48.0 μM[b]	58
		Penochalasin A **10**	Alkaloid	KB cell line	40.0 μM[b]	58
Cynodon dactylon	*Aspergillus fumigatus*	9-Deacetoxyfumigaclavine **11**	Alkaloid	K562	3.10 μM[b]	59
Mediterranean green alga	*Emericella nidulans*	Emindole DA **12**	Alkaloid	36 human tumor	5.5 μg/mL[b]	31
Tripterygium wilfordii	*Rhinocladiella* sp.	Cytochalasin 1 **13**[a]	Alkaloid	A2780S, HCT-116, SW-620	3.91, 15.6, 3.91 μg/mL[c]	61
		Cytochalasin 2 **14**[a]	Alkaloid	A2780S, HCT-116, SW-620	15.6, 62.5, 15.6 μg/mL[c]	61
		Cytochalasin 3 **15**[a]	Alkaloid	A2780S, SW-620	3.91, 15.6 μg/mL[c]	61
		Cytochalasin E **16**	Alkaloid	A2780S, HCT-116	<0.015, 0.98 μg/mL[c]	61
				SW-620	0.244 μg/mL[c]	61
Ulva pertusa	*Chaetomium globosum*	Cytoglobosin C **17**[a]	Alkaloid	A549	2.26 μM[b]	63
		Cytoglobosin D **18**[a]	Alkaloid	A549	2.55 μM[b]	63
Adenophora axilliflora	*Chaetomium* sp. IFB-E015	Chaetominine **19**[a]	Alkaloid	K562, SW1116	21.0, 28.0 nM[b]	64
Artemisia annua	*Hypoxylon truncatum*	Daldinone C **20**[a]	Benzo[*j*]fluoranthene	SW1116	49.5 μM[b]	65
		Daldinone D **21**[a]	Benzo[*j*]fluoranthene	SW1116	41.0 μM[b]	65
Rhizophora mucronata	*Pestalotiopsis* sp.	Pestalotiopsone F **22**[a]	Chromone	L5178Y	8.93 μg/mL[d]	66
Camellia sinensis	*Pestalotiopsis fici*	Pestaloficiol I **23**[a]	Chromone	HeLa, MCF7	>136.1, 136.1 μM[b]	67
		Pestaloficiol J **24**[a]	Chromone	HeLa, MCF7	21.2, >153.8 μM[b]	67
		Pestaloficiol K**25**[a]	Chromone	HeLa, MCF7	99.3, >132.5 μM[b]	67
		Pestaloficiol L **26**[a]	Chromone	HeLa, MCF7	8.7, 17.4 μM[b]	67
Polysiphonia violacea	*Apiospora montagnei*	Epiepoxydon **27**	Cyclohexanone	HM02	0.70 μg/mL[e]	71
				HepG2	0.75 μg/mL[e]	71
				MCF7	0.8 μg/mL[e]	71
Leaf, Hala-Bala Forest	BCC 8616	Depsidone 1 **28**[a]	Depsidone	KB, BC	6.5, 4.1 μg/mL[b]	72
Ephedra fasciculata	*Fusarium oxysporum*	Beauvericin **29**	Depsipeptide	NCI-H460	1.41 μM[b]	73
				MIA Pa Ca-2	1.66 μM[b]	73
				MCF-7, SF-268	1.81, 2.29 μM[b]	73
Mimosops elengi	PM0651480	Ergoflavin **30**	Ergochrome	TNF-α	1.9 ± 0.1.μM[b]	77
				IL-6	1.2 ± 0.3 μM[b]	77
				ACHN, H-460	1.2, 4.0 μM[b]	77
				Panc1, HCT116	2.4, 8.0 μM[b]	77
				Calu1	1.5 μM[b]	77
Dicerandra frutescens	*Phomopsis longicolla*	Dicerandrol A **31**[a]	Ergochrome	A549, HCT-116	7.0, 7.0 μg/mL[c]	78
		Dicerandrol B **32**[a]	Ergochrome	A549, HCT-116	1.8, 1.8 μg/mL[c]	78
		Dicerandrol C **33**[a]	Ergochrome	A549, HCT-116	1.8, 7.0 μg/mL[c]	78
Mangrove plant	ZSU44	Secalonic acid D **34**	Ergochrome	HL-60, K562	0.38, 0.43 μM[b]	81

Table 1 (*Contd.*)

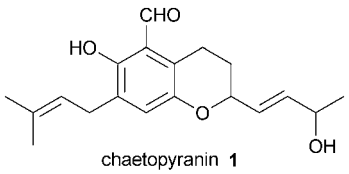
Host	Fungal endophyte	Compound	Chemical nature	Cell line/Target enzyme	Activity	Ref.
Ephedra fasciculata	*Chaetomium globosum*	Globosumone A **35**[a]	Ester	NCI-H460, MCF-7	6.50, 21.30 μM[b]	16
				SF-268, WI-38	8.80, 13.00 μM[b]	16
				MIA Pa Ca-2	10.60 μM[b]	16
		Globosumone B **36**[a]	Ester	NCI-H460, MCF-7	24.80, 21.90 μM[b]	16
				SF-268, WI-38	29.10, 14.20 μM[b]	16
				MIA Pa Ca-2	30.20 μM[b]	16
Taxus mairei & *Torreya grandis*	*Aspergillus clavatus* & *Paecilomyces* sp.	Brefeldin A **37**	Lactone	HL-60	10.0 ng/mL[b]	83
				KB	9.0 ng/mL[b]	83
				MCF-7	2.0 ng/mL[b]	83
				Spc-A-1	1.0 ng/mL[b]	83
				HeLa	1.8 ng/mL[b]	83
Knema laurina	*Acremonium* sp.	Brefeldin A **37**	Lactone	KB, BC-1	0.18, 0.04 μM[b]	84
				NCI-H187	0.11 μM[b]	84
Ephedra fasciculata	*Chaetomium chiversii*	Radicicol **38**	Lactone	MCF-7	0.03 μM[b]	85
Roystonea regia	*Pestalotiopsis photiniae*	Photinides A–F **39–44**[a]	Lactone	MDA-MB-231	10 μg/mL[g]	88
Etlingera littoralis	*Eutypella* sp.	Eutypellin A **45**[a]	Lactone	NCI-H187, MCF-7	12, 84 μM[b]	89
				KB, Vero	38, 88 μM[b]	89
Podophyllum hexandrum	*Trametes hirsuta*	Podophyllotoxin **46**	Lignan	Topoisomerase I	—	48
Podophyllum peltatum	*Phialocephala fortinii*	Podophyllotoxin	Lignan	Topoisomerase I	—	49
Taxus baccata	*Acremonium* sp.	Leucinostatin A **47**	Peptide	BT-20	2.0 nM[f]	94
Musa acuminata	*Phomopsis* sp.	Oblongolide Y **49**[a]	Polyketide	BC	48 μM[b]	95
		Oblongolide Z **50**[a]	Polyketide	KB, BC,	37.0, 26.0 μM[b]	95
				NCI-H187	32.0 μM[b]	95
				Vero cells	60 μM[b]	95
Polygonum senegalense	*Alternaria* sp.	Alternariol **51**	Polyketide	L5178Y	1.7μg/mL[d]	96
		Alternariol 5-*O*-sulfate **52**[a]	Polyketide	L5178Y	4.5 μg/mL[d]	96
		Alternariol 5-*O*-methyl ether **53**	Polyketide	L5178Y	7.8 μg/mL[d]	96
Polygonum senegalense	*Alternaria* sp.	Altenusin **54**	Polyketide	L5178Y	6.8 μg/mL[d]	96
		Desmethylaltenusin **55**[a]	Polyketide	L5178Y	6.2 μg/mL[d]	96
Aegiceras corniculatum	*Penicillium* sp.	Leptosphaerone C **56**[a]	Polyketide	A549	1.45 μM[b]	29
		Penicillenone **57**[a]	Polyketide	P388	1.38 μM[b]	29
Excoecaria agallocha	*Phomopsis* sp	2-(7′-Hydroxyoxooctyl)-3-hydroxy-5-methoxybenzene acetic acid ethyl ester **58**[a]	Polyketide	HEp-2 & HepG2	25 and 30 μg/mL[b]	97
Mediterranean green alga	*Emericella nidulans* var. *acristata*	Arugosin A **59**	Polyketide	7 out of 36	10 μg/mL[b]	31
		Arugosin B **60**	Polyketide	Human tumor	—	31
Cylindropuntia echinocarpus	*Fusarium oxysporum*	Bikaverin **61**	Polyketide	NCI-H460	0.43 μM[b]	73
				MIA Pa Ca-2	0.26 μM[b]	73
				MCF-7, SF-268	0.42, 0.38 μM[b]	73
Sequoia sempervirens	*Aspergillus parasiticus*	Sequoiatones A **62**[a]	Polyketide	BC	4–10 μM[e]	98
Sequoia sempervirens	*Aspergillus parasiticus*	Sequoiatones B **63**[a]	Polyketide	BC	4–10 μM[e]	98
Sequoia sempervirens	*Aspergillus parasiticus*	Sequoiamonascin A **64**[a]	Polyketide	MCF7	1% cell growth[h]	99
				NCI-H460	1% cell growth[h]	99
				SF-268 (CNS)	2% cell growth[h]	99
Sequoia sempervirens	*Aspergillus parasiticus*	Sequoiamonascin B **65**[a]	Polyketide	MCF7	19% cell growth[h]	99
				NCI-H460	4% cell growth[h]	99
				SF-268 (CNS)	15% cell growth[h]	99
Seaweed	*Talaromyces* sp.	Kasanosin A **66**[a]	Polyketide	DNA pol β, γ	27.3 μM, 35.0 μM[b]	100
		Kasanosin B **67**[a]	Polyketide	DNA pol β, γ	60.1 μM, 72.9 μM[b]	100
Hypericum perforatum	*Thielavia subthermophila*	Hypericin **68**	Polyketide	THP-1	fungal extract, cell viability-1.0% (light)	109

Table 1 (*Contd.*)

Host	Fungal endophyte	Compound	Chemical nature	Cell line/Target enzyme	Activity	Ref.
Hypericum perforatum	*Thielavia subthermophila*	Emodin **69**	Polyketide	THP-1	fungal extract, cell viability-1.0% (light)	109
Cynodon dactylon	*Aspergillus niger*	Rubrofusarin B **70**	Polyketide	SW1116	4.5 μg/mL[b]	110
Torreya taxifolia	*Pestalotiopsis microspora*	Torreyanic acid **71**[a]	Quinone	NEC, A549	3.5, 45.0 μg/mL[b]	111
				25 tumor cell lines	9.5 μg/mL (mean)[b]	111
Mentha pulegium	*Stemphylium globuliferum*	Mixture of alterporriol G & alterporriol H **72**[a]	Quinone	L5178Y	2.7 μg/mL[d]	112
		6-*O*-Methylalaternin **73**	Quinone	L5178Y	4.2 μg/mL[d]	112
Salvia officinalis	*Chaetomium* sp.	Cochliodinol **74**	Quinone	L5178Y	7.0 μg/mL[d]	113
		Isocochliodinol **75**	Quinone	L5178Y	71.5 μg/mL[d]	113
Sandoricum koetjape	*Xylaria* sp.	2-Chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione **76**[a]	Quinone	Vero cells	1.35 μM[b]	115
		Xylariaquinone A **77**[a]	Quinone	Vero cells	>184 μM[b]	115
Mangrove plant	*Halorosellinia* sp. & *Guignardia* sp.	Anthracenedione 1 **78**	Quinone	KB, KBv200	57.32, 90.86 μM[b]	118
		Anthracenedione 5 **79**	Quinone	KBv200	86.45 μM[b]	118
		Anthracenedione 6 **80**	Quinone	KB, KBv200	3.17, 3.21 μM[b]	118
		Anthracenedione 7 **81**	Quinone	KB	56.56 μM[b]	118
		Anthracenedione 9 **82**	Quinone	KB, KBv200	38.05, 34.64 μM[b]	118
		Anthracenedione 14 **83**	Quinone	KB	68.39 μM[b]	118
Knightia excelsa	Mycelia sterilia	Spiromamakone A **84**[a]	Spirobisnaphthalene	P388	0.33 μM[b]	119
Aquilaria sinensis	*Preussia* sp.	Spiropreussione A **85**[a]	Spirobisnaphthalene	A2780	2.4 μM[b]	120
				BEL-7404	3.0 μM[b]	120
Xylopia aromatica	*Periconia atropurpurea*	Periconicin B **86**[a]	Terpene	HeLa and CHO	8.0, 8.0 μM[b]	121
Taxus brevifolia	*Taxomyces andreanae*	Paclitaxel **87**	Terpene	—	—	12
Taxus wallichiana	*Pestalotiopsis microspora*	Paclitaxel **87**	Terpene	—	—	12
Terminalia arjuna	*Pestalotiopsis terminaliae*	Paclitaxel **87**	Terpene	BT220, H116, INT-407, HL251, HLK210	0.005–5 μM	129
Aegle marmelos	*Bartalinia robillardoides*	Paclitaxel **87**	Terpene	—	—	131
Etlingera littoralis	*Eutypella* sp.	*ent*-4(15)-Eudesmen-11-ol-1-one **88**[a]	Terpene	NCI-H187, MCF7	11, 20 μM[b]	89
				KB and Vero	32, 32 μM[b]	89
Knema laurina	KLAR 5	8-Deoxy-trichothecin **89**	Terpene	BC-1, NCI-H187	0.88, 1.48 μM[b]	84
		7α-Hydroxytrichodermol **91**	Terpene	BC-1, NCI-H187	2.37, 1.73 μM[b]	84
		Trichothecolone **90**	Terpene	KB, BC-1	12.90, 10.06 μM[b]	84
				NCI-H187	11.31 μM[b]	84
		7α-Hydroxyscirpene **92**	Terpene	KB, BC-1	8.47, 21.53 μM[b]	84
				NCI-H187	27.76 μM[b]	84
Platycladus orientalis	*Phyllosticta spinarum*	Tauranin **93**	Terpene	NCI-H460, PC-3M	4.3, 3.5 μM[b]	132
				MCF-7, SF-268	1.5, 1.8 μM[b]	132
				MIA Pa Ca-2	2.8 μM[b]	132
Xylocarpus granatum	XG8D (a basidiomycete)	Merulin A **94**[a]	Terpene	BT474, SW620	4.98, 4.84 μg/mL[b]	133
		Merulin C **95**[a]	Terpene	BT474, SW620	1.57, 4.11 μg/mL[b]	133
Licuala spinosa	*Xylaria* sp.	Eremophilanolides 1–3 **96–98**[a]	Terpene	KB, MCF-7, NCI-H187	3.8–21 μM[b]	134
Tectona grandis	*Phomopsis* sp.	Phomoxanthone A **99**[a]	Xanthone	KB, BC-1, Vero	0.99, 0.51, 1.4 μg/mL[b]	135
		Phomoxanthone B **100**[a]	Xanthone	KB, BC-1, Vero	4.1, 0.70, 1.8 μg/mL[b]	135

[a] Novel compound. [b] IC_{50}. [c] IC_{100}. [d] EC_{50}. [e] IG_{50}. [f] LD_{50}. [g] IG_{25}. [h] 10 μM.

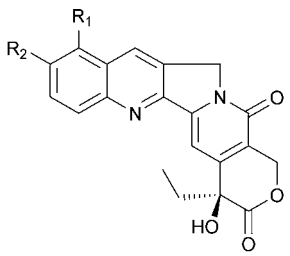
microvascular endothelial cells), SMMC-7721 (hepatocellular carcinoma cells) and A549 (human lung epithelial cells) with IC_{50} values of 15.4, 28.5, and 39.1 μg/mL respectively. Chaetopyranin **1** was also evaluated for its radical scavenging abilities using DPPH (1,1-diphenyl-2-picrylhydrazyl). Compound **1** showed moderate activity with an IC_{50} value of 35 μg/mL, compared to an IC_{50} value of 18 μg/mL for the positive control BHT (butylated hydroxytoluene).[27]



chaetopyranin **1**

3.3.2 Alkaloids and nitrogen-containing heterocycles. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms. Not all nitrogen-containing compounds are basic, however, and the distinction can be subtle. For this reason, nitrogen-containing compounds are included as a single group. Plant-derived alkaloids exhibit biological activities that range from toxic to medicinal to recreational – and sometimes these activities overlap. Many plant alkaloids have been studied as potential anticancer agents. It has been of great interest that some of the most potent of these plant-derived antitumor alkaloids have also been reported as isolates from endophytic fungi. These endophytes have usually been associated with a host organism that has also been reported to produce the compound of interest.

Camptothecin **2** (CPT) is a pentacyclic quinoline alkaloid that inhibits topoisomerase I (topo I), an enzyme involved in DNA replication. The compound acts as a potent antineoplastic agent, and exerts its cytotoxic effect by inhibiting the dissociation of the DNA–topoisomerase I complex during replication.[50,51] Camptothecin and its derivatives are unique for a number of reasons. Topoisomerase I is apparently the only enzyme target of these alkaloids. Yeast cell mutants lacking topoisomerase I were immune to the cytotoxic effects of CPT.[52] Similarly vertebrate cell lines selected for CPT resistance exhibited mutations in topo I.[53] CPT penetrates vertebrate cells readily and targets topo I within minutes of exposure to low or even submicromolar drug concentrations. It does not bind to DNA or to topo I independently, but only to the complex formed by topo I when it cleaves DNA.[54]



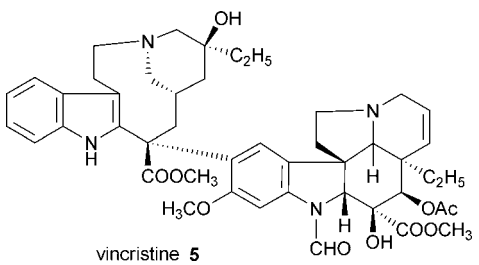
R_1, R_2 = H camptothecin **2**
R_2 = H; R_1 = OCH_3 9-methoxycamptothecin **3**
R_1 = H; R_2 = OH 10-hydroxycamptothecin **4**

Camptothecin was initially isolated from the wood of *Camptotheca acuminata* (Nyssaceae) a plant native to mainland China that exhibited potent antileukemic and antitumor activities in animals.[54] Called '*xi shu*' or the 'happy tree', it has a long history of medicinal use in China. It is interesting to note that this complex alkaloid and its analogue 9-methoxycamptothecin have been isolated from the inner bark[55] and from cell suspension cultures of *Nothapodytes foetida*, a small evergreen tree found in southern India and Sri Lanka that is unrelated to *Camptotheca*.[56,57]

In recent years, however, camptothecin has been isolated from fungal endophytes of both of these plants. Camptothecin was isolated in 2005 from a fungal endophyte isolated from the inner bark of *Nothapodytes foetida* identified as *Entrophosphora infrequens*[42] based on the molecular analysis of a fragment of the large ribosomal subunit gene (LSU). It had an identity of 99.8% with *E. infrequens* and a 98.6% identity index with certain strains of *Rhizopus oryzae*.[42] Three years later CPT was isolated from a *C. acuminata* seed endophyte, *Neurospora crassa*.[43] Both authentic CPT and fungal CPT were tested against human cancer cell lines A549 (lung cancer), HEP-2 (liver cancer), and OVCAR-5 (ovarian cancer) with comparable results.[43] The following year camptothecin and two of its analogues, 9-methoxycamptothecin **3** and 10-hydroxycamptothecin **4**, were isolated from *Fusarium solani*, an endophytic fungus of *Camptotheca acuminata*.[44] Both analogues are more water-soluble than camptothecin and more potent inhibitors of DNA topoisomerase I.[44]

Although camptothecin itself is not used as a drug, two water-soluble derivatives of the parent camptothecin are among the most recently FDA-approved anticancer agents. Camptosar® (irinotecan hydrochloride) has been approved for the treatment of colorectal carcinomas, and Hycamtin® (topotecan), the first orally available CPT derivative, has been approved for the treatment of ovarian cancers and non-small-cell lung cancers. It has also been approved for the treatment of cervical cancer when used in conjunction with cisplatin.

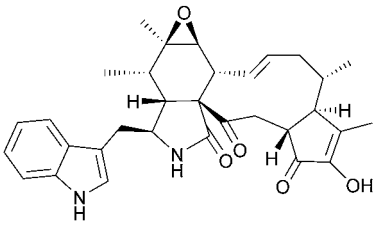
Vincristine **5** (Oncovin®), also known as leurocristine, is a vinca alkaloid originally isolated from *Catharanthus roseus*,[6] a member of the family Apocyanaceae. Among its many activities in cellular systems, vincristine binds irreversibly to both microtubules and spindle proteins in the S phase of the cell cycle. It interferes with the formation of the mitotic spindle and consequently arrests tumor cells in the metaphase. It has been isolated by different researchers from the *Catharanthus roseus* endophyte *Fusarium oxysporum*.[45–47]



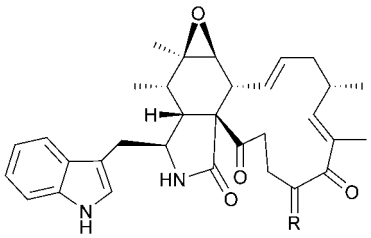
vincristine **5**

Chaetoglobosin U **6** is a cytochalasin-based alkaloid isolated from *Chaetomium globosum* IFB-E019, an endophytic fungus residing within the stem of healthy *Imperata cylindrica*. It exhibited cytotoxic activity against the human nasopharyngeal epidermoid tumor KB cell line with an IC_{50} value of 16.0 μM comparable to that of 5-fluorouracil co-assayed as a positive reference (14.0 μM). The four previously isolated analogues of

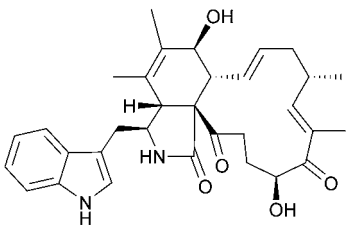
chaetoglobosin U, named chaetoglobosins C **7**, F **8**, E **9** and penochalasin A **10**, showed moderate activity against the same cell line, with IC_{50} values of 34.0, 52.0, 48.0, and 40.0 μM, respectively.[58]



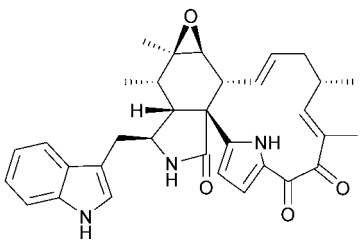
chaetoglobosin U **6**



R = O chaetoglobosin C **7**
R = H,α-OH chaetoglobosin F **8**



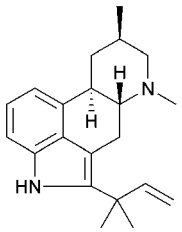
chaetoglobosin E **9**



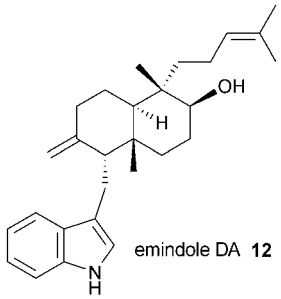
penochalasin A **10**

9-Deacetoxyfumigaclavine C **11** was isolated from the endophyte *Aspergillus fumigatus*, which was obtained from a healthy stem of *Cynodon dactylon*. It exhibited potent cytotoxicity against human leukemia cells (K562) with an IC_{50} value of 3.1 μM, which was similar to that of doxorubicin hydrochloride (1.2 μM), a drug which is currently used for the treatment of leukemia.[59]

Indole alkaloid emindole DA **12** was isolated from *Emericella nidulans* var. *acristata*, an endophyte of a unspecified Mediterranean green alga.[31] It exhibited antitumor activity against 36 human tumor cell lines representing 11 different tumor types, with a mean IC_{50} value of 5.5 μg/mL compared to the reference compound adriamycin tested in parallel in the same assays with an IC_{50} value of 0.16 μg/mL.[31]

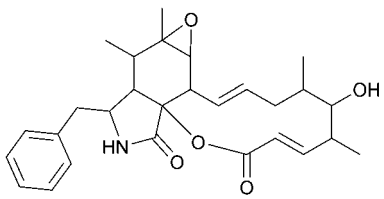


9-deacetoxyfumigaclavine C **11**

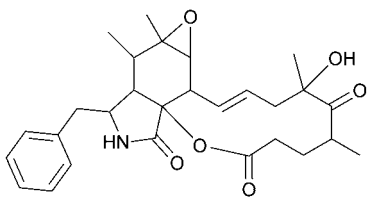


emindole DA **12**

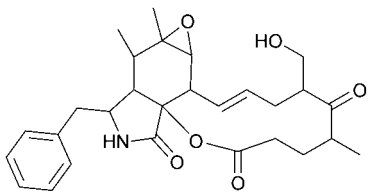
Cytochalasins are a class of fungal metabolites characterized by a highly substituted perhydroisoindol-1-one moiety usually fused to either an 11- or 13-membered macrocyclic ring. The class exhibits a wide range of biological activities and has been isolated from many different fungal genera. To date, more than 80 different cytochalasins have been reported from fungi isolated from soil or marine sediments. Fungal endophytes, however, have contributed four novel members to this class of molecules. Cytochalasins **13**, **14**, **15** and **16** have been reported as cytotoxic agents from the endophytic fungus *Rhinocladiella* sp. associated with the perennial twining vine *Tripterygium wilfordii*.[60] Compound **16** was previously reported from this same fungal isolate.[61] These compounds were identified as 22-oxa[12]cytochalasins and were tested against three different tumor cell



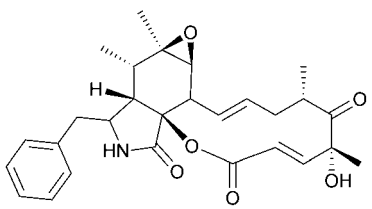
cytochalasin 1 **13**



cytochalasin 2 **14**



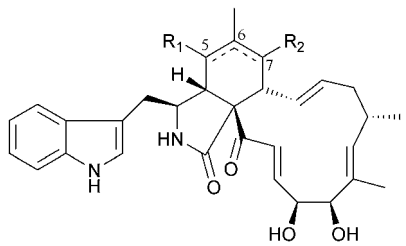
cytochalasin 3 **15**



cytochalasin E **16**

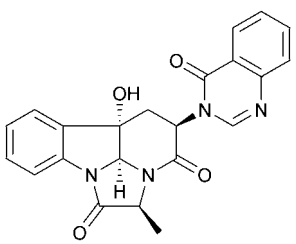
lines: A2780S (ovarian tumor cell line), HCT-116 (colon tumor cell line and SW-620 (colon tumor cell line). Cytochalasin **13** exhibited IC_{100} values of 3.91, 15.6 and 3.91 μg/mL respectively, while **14** exhibited values of 15.6, 62.5, 15.6 μg/mL respectively. Cytochalasin **15** did not show any activity against the HCT-116 cell line, and exhibited an IC_{100} value of 3.91 μg/mL against A2780S and 15.6 μg/mL against SW-620.[60] Cytochalasin **16** exhibited the greatest potency against these cell lines, with IC_{100} values of <0.0153, 0.977 and 0.244 μg/mL respectively. Cytochalasins are known to induce apoptosis by inhibiting cell division due to their ability to bind with, and inhibit the polymerization of, actin filaments.[62]

Two novel fungal alkaloids, cytoglobosins C **17** and D **18**, were isolated and identified from cultures of *Chaetomium globosum* QEN-14, an endophytic fungus isolated from the marine green alga *Ulva pertusa*. The compounds displayed very similar cytotoxicity profiles, with IC_{50} values of 2.26 and 2.55 μM against the A549 tumor cell line.[63]



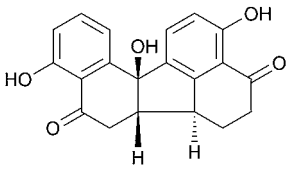
R_1 = Me, R_2 = β-OH, Δ5,6 cytoglobosin C **17**
R_1 = α-Me, R_2 = H, Δ6,7 cytoglobosin D **18**

Chaetomium sp. IFB-E015, an endophytic fungus on apparently healthy *Adenophora axilliflora* leaves, produced an alkaloid, chaetominine **19**, which was cytotoxic against the human leukemia K562 and colon cancer SW1116 cell lines with corresponding IC_{50} values of 21.0 and 28.0 nM. Its potency was greater than that of 5-fluorouracil, with IC_{50} values of 33.0 and 76.0 nM, respectively.[64]

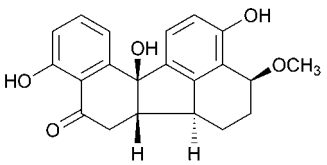


chaetominine **19**

3.3.3 Benzo[*j*]fluoranthenes. Daldinone C **20** and daldinone D **21** were isolated from *Hypoxylon truncatum* IFB-18, an endophyte of *Artemisia annua*. Both compounds exhibited potent cytotoxicity against SW1116 cells (human colorectal cancer cell line), with IC_{50} values of 49.5 and 41.0μM respectively, comparable to that of 5-fluorouracil (37.0 μM).[65] It is interesting to note that the same researchers using the same cell line (SW1116) and the same MTT colorimetric method had disparate cytotoxicity values for 5-fluorouracil. In their report of the isolation of chaetominine,[64] they reported a value of 76.0 nM, whereas in this study they reported a value of 37 μM for 5-fluorouracil.[65]

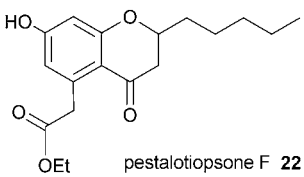


daldinone C **20**



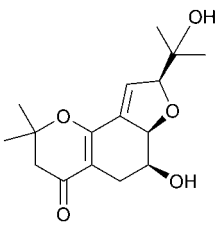
daldinone D **21**

3.3.4 Chromones. Pestalotiopsone F **22** (5-carbomethoxymethyl-7-hydroxy-2-pentylchromone) is a novel chromone isolated from the fungus *Pestalotiopsis* sp., an endophyte of the Chinese mangrove plant *Rhizophora mucronata*.[66] Compound **22** displayed moderate cytotoxicity against the murine cancer cell line L5178Y, with an EC_{50} value of 8.93 μg/mL.[66]

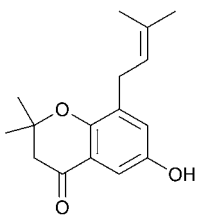


pestalotiopsone F **22**

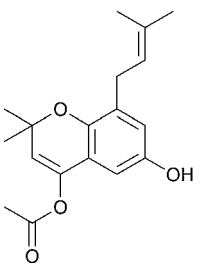
Four novel isoprenylated chromone derivatives, pestaloficiol I **23**, pestaloficiol J **24**, pestaloficiol K **25** and pestaloficiol L **26** (heterodimer), were isolated from *Pestalotiopsis fici*, a fungal endophyte of *Camellia sinensis*. The IC_{50} values of the 4 compounds ranged between 8.7 μM and >136.1 μM for HeLa cells and between 17.4 μM and >153.8 μM for MCF7 cells, compared to the positive control 5-fluorouracil with IC_{50} values of 10.0 and 15.0 μM, respectively.[67] Compound **26** exhibited the most potent cytotoxicity, with IC_{50} values of 8.7 and 17.4 μM respectively.



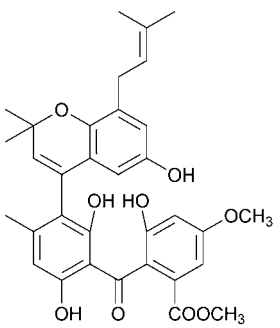
pestaloficiol I **23**



pestaloficiol J **24**



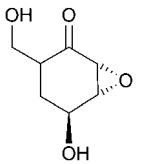
pestaloficiol K **25**



pestaloficiol L **26**

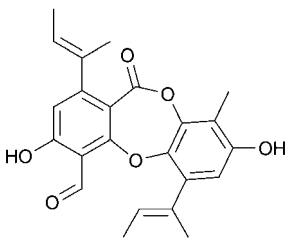
3.3.5 Cyclohexanones. The known compound epiepoxydon **27**[68–70] was isolated from a marine endophyte, *Apiospora montagnei* of the North Sea alga *Polysiphonia violacea*.[71] In the brine shrimp assay the compound was strongly cytotoxic. It exhibited an LC_{50} of 3.6 μg/mL for the breast adenocarcinoma cell line

MCF7 and GI_{50} concentrations of 0.7 μg/mL for the human gastric carcinoma HM02, 0.75 μg/mL for the human liver carcinoma HepG2, and 0.8 μg/mL for MCF7. Total growth inhibition (TGI) for these cell lines was also determined and was found to be 1.0 μg/mL for HM02, 4.6 μg/mL for HepG2, and 1.5 μg/mL for MCF7. In the case of HM02 and HepG2 cells the LC_{50} of compound **27** was >10 μg/mL. Compound **27** was previously reported to have an ED_{50} of 0.2 μg/mL towards the P388 lymphocytic leukemia cell line.[68]



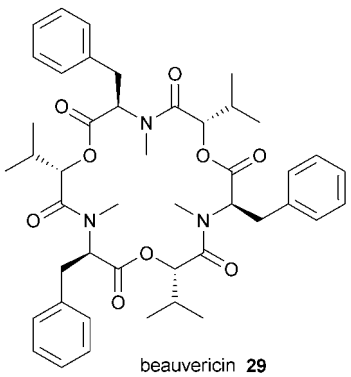
(+)-epiepoxydon **27**

3.3.6 Depsidones. Depsidone 1 **28** was isolated from an endophytic fungus of the order Pleosporales (BCC 8616) that was isolated from an unidentified leaf of the Hala-Bala evergreen forest.[72] Depsidone 1 **28** exhibited weak cytotoxic activity against KB and BC cell lines, with IC_{50} values of 6.5 and 4.1 μg/mL, respectively.[72]



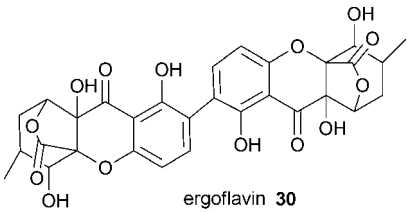
depsidone 1 **28**

3.3.7 Depsipeptides. Beauvericin **29** is a depsipeptide isolated from *Fusarium oxysporum* EPH2RAA, an endophytic fungus of the Sonoran desert plant *Ephedra fasciculata*.[73] It has previously been isolated from several other fungi.[74–76] Beauvericin exhibited cytotoxic activity against four different cell lines, NCI-H460 (human non-small-cell lung cancer), MIA Pa Ca-2 (human pancreatic carcinoma), MCF-7 (human breast cancer) and SF-268 human CNS cancer (glioma) with IG_{50} values of 1.41, 1.66, 1.81 and 2.29 μM, respectively, compared to the standard compound doxorubicin with values of 0.01, 0.05, 0.07 and 0.04 μM respectively.[73]



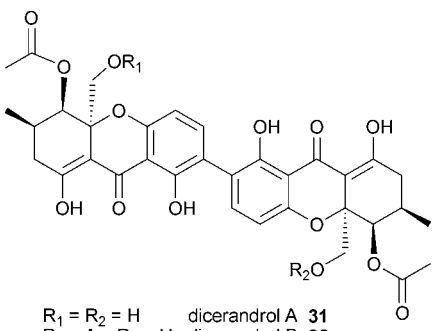
beauvericin **29**

3.3.8 Ergochromes. Ergoflavin **30** is a member of the class of compounds called ergochromes which are dimeric xanthenes linked in position 2. These compounds were first isolated from the ergot fungus *Claviceps purpurea*, as well as *Phoma terrestris*, *Pyrenochaeta terrestris*, *Penicillium oxalicum*, and *Aspergillus* sp.[77] It has been isolated from a leaf ascomycetous endophyte of *Mimosops elengi* (‘*bakul*’) designated PM0651480. Ergoflavin significantly inhibited human TNF-α and IL-6, with IC_{50} values of 1.9 ± 0.1 and 1.2 ± 0.3 μM compared to dexamethasone, with IC_{50} values of 0.06 ± 0.007 and 0.01 ± 0.0 μM for TNF-α and IL-6 inhibition, respectively.[77] It also exhibited cytotoxicity against the following human cancer cell lines: renal ACHN, lung H460, pancreatic Panc1, colorectal HCT116, and lung Calu1 cancer cell lines, with IC_{50} values of 1.2 ± 0.20, 4.0 ± 0.08, 2.4 ± 0.02, 8.0 ± 0.45, and 1.5 ± 0.21 μM, respectively. Flavopiridol, a known anticancer compound, was used as a standard for evaluating the cytotoxicity of ergoflavin with IC_{50} values in the following cancer cells: ACHN, 0.84 ± 0.03 μM; H460, 0.38 ± 0.01 μM; Panc-1, 0.23 ± 0.07 μM; HCT116, 0.25 ± 0.03 μM; and Calu1, 0.41 ± 0.09 μM.[77]



ergoflavin **30**

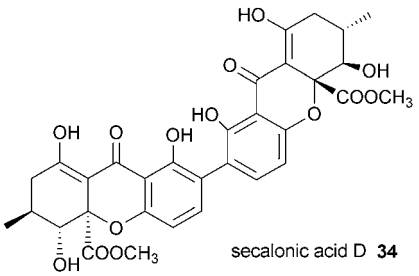
Phomopsis longicola is an endophytic fungus of the rare mint *Dicerandra frutescens*.[78] *D. frutescens* is found in only a dozen sites within a few hundred acres in central Florida. The plant is on the Federal Endangered Species List, but has been the subject of much study due to its rich chemistry.[79,80] The mint is virtually untouched by insect predators, and earlier studies found that the monoterpenes in the leaves are effective insect deterrents.[79,80] The fungal endophyte produced three compounds designated dicerandrols A **31**, B **32**, and C **33**, which have been classified as ergochromes because they have the same tricyclic C_{15} system with a similar arrangement of substituents.[78] The dicerandrols exhibited significant cytoxicity against two human cancer cell lines, lung adenocarcinoma epithelial cell line A549 and colorectal HCT-116. The IC_{100} value of **31** against both cell lines and the value of **33** against HCT-116 was 7.0 μg/mL. The IC_{100} value of **33** against A549 and of **32** against both cell lines was



$R_1 = R_2 = H$ dicerandrol A **31**
$R_1 = Ac, R_2 = H$ dicerandrol B **32**
$R_1 = R_2 = Ac$ dicerandrol C **33**

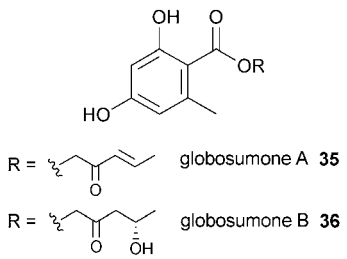
1.8 μg/mL. These values are significantly better than the standard anticancer drug etoposide, which has IC_{100} values of 30.0 μg/mL against A549 and 125.0 μg/mL against HCT-116.[78]

Secalonic acid D **34** was isolated from the mangrove endophytic fungus no. ZSU44.[81] It was first isolated in 1970 from *Penicillium oxalicum*[82] and was found to be extremely toxic and teratogenic.[81] Secalonic acid D showed potent cytotoxicity to HL60 and K562 cells, with IC_{50} values of 0.38 and 0.43 μM, respectively. Further testing with the Annexin V-FITC/PI assay and Western blot indicated that secalonic acid D induced apoptosis in HL60 and K562 cells. Secalonic acid D also led to cell cycle arrest of G1 phase related to downregulation of c-Myc.[81]



secalonic acid D **34**

3.3.9 Esters. Globosumone A **35** and B **36** are orsellinic acid esters isolated from a well-studied endophytic fungus, *Chaetomium globosum* isolated from Mormon tea, *Ephedra fasciculata*.[16] Both compounds exhibited cytotoxic activity against NCI-H460 (non-small-cell lung cancer), MCF-7 (breast cancer), SF-268 (CNS glioma), and MIA Pa Ca-2 (pancreatic carcinoma) and WI-38 normal human fibroblast cells (Table 2).[16]



globosumone A **35**

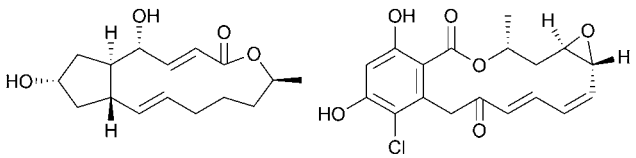
globosumone B **36**

Table 2 Cytotoxicities (IC_{50}/μM) of globosumone A **35** and B **36** against four human cancer cell lines and normal human fibroblast cells.[16]

Compound	NCI-H460	MCF-7	SF-268	MIA Pa Ca-2	WI-38
35	6.50	21.30	8.80	10.60	13.00
36	24.80	21.90	29.10	30.20	14.20

3.3.10 Lactones. Brefeldin A **37** has been isolated from several fungal species including *Curvularia*, *Alternaria*, *Ascochyta*, *Phyllosticta*, *Penicillium*, and *Cercospora*.[83] The compound has antifungal, anticancer and antiviral activities. Brefeldin A **37** was isolated from two different endophytic fungi, *Aspergillus clavatus* and *Paecilomyces* sp., which were isolated from the tissues of Chinese *Taxus mairei* and *Torreya grandis*.[83] Compound **37** showed strong cytotoxicity against HL-60, KB, HeLa, MCF-7 and Spc-A-1 cell lines, with IC_{50} values of 10.0, 9.0, 1.8, 2.0 and 1.0 ng/mL, compared to the standard anticancer compound paclitaxel, which had IC_{50} values of 1.2, 0.16, 1.8, 5.0 and 0.8 ng/mL, respectively.[83]

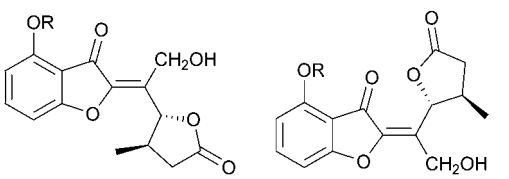
Brefeldin A **37** was also isolated from a new species of *Acremonium* which was isolated from a healthy twig of the Thai medicinal plant *Knema laurina*.[84] In this study, **37** showed potent activity against the following human cancer cell lines: KB (epidermoid cancer of the mouth), BC-1 (breast cancer) and NCI-H187 (small-cell lung cancer), with IC_{50} values of 0.18, 0.04, and 0.11 μM, respectively.[84]



brefeldin A **37** radicicol **38**

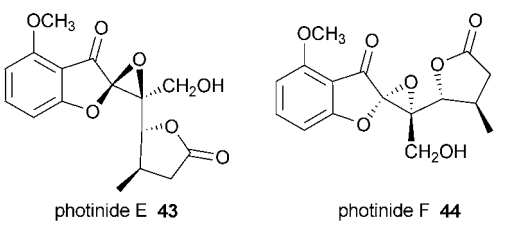
The known compound radicicol **38** was isolated from *Chaetomium chiversii*, an endophytic fungus of *Ephedra fasciculata*, as part of an ongoing investigation of the endophytes of Sonoran desert plants and of inhibitors of HSP90 (heat shock protein).[85] Hsp90 has dual roles in the stress response and in maintaining regulatory signaling networks.[86] Hsp90 may play a critical role in the cancer phenotype, and thus provide an effective target for cancer chemotherapy. Cancer cells frequently express high levels of Hsp90, presumably in response to the stress conditions within the tumor microenvironment. As a result, pharmacological inhibition of this single target by compounds such as geldanamycin has been shown to simultaneously destabilize many of the substrates known to be critical for the process of multistep carcinogenesis.[87] Radicicol **38** also exhibited antiproliferative activity against breast cancer cell line MCF-7, with an IC_{50} value of 0.03 μM.[85]

Six novel benzofuranone-derived γ-lactones, photinides A–F **39–44**, were isolated from *Pestalotiopsis photiniae*, an endophyte of *Roystonea regia*.[88] All six γ-lactones exhibited cytotoxicity against breast cancer cell line MDA-MB-231 with inhibitory rates of 24.4%, 24.2%, 23.1%, 24.4%, and 24.6%, respectively, at a concentration of 10 μg/mL.[88]



R = CH_3 photinide A **39**
R = H photinide C **40**

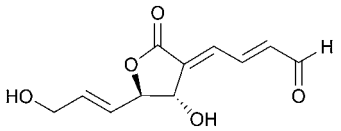
R = CH_3 photinide B **41**
R = H photinide D **42**



photinide E **43** photinide F **44**

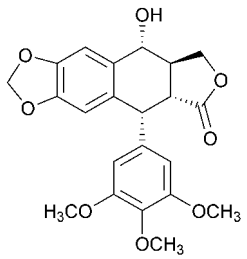
Eutypellin A **45** is a γ-lactone that exhibited cytotoxic activity against NCI-H187 (human small-cell lung cancer cells), MCF-7,

KB and non-malignant Vero cells with IC_{50} values of 12, 84, 38 and 88 μM compared to the standard ellipticine, which exhibited IC_{50} values of 3.6, 2.5 and 5.5 μM respectively.[89] Eutypellin A was isolated from the endophytic fungus *Eutypella* sp. BCC 13199, itself isolated from *Etlingera littoralis* (Earth ginger).[89]



eutypellin A **45**

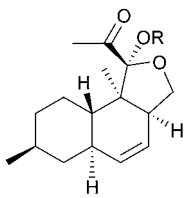
3.3.11 Lignans. The aryltetralin lignan podophyllotoxin **46** is an important natural product which was originally isolated in 1950 from the higher plant *Podophyllum emodi*.[90] Compound **46** is currently used as a treatment for genital warts,[91] but its greater value is its role as the precursor to three anticancer drugs, the topoisomerase I inhibitors etoposide, teniposide, and etoposide phosphate.[48,49] The difficulties involved in total synthesis of these lignans, as well as the destruction of wild populations of the primary source plant, have led many researchers to search for alternative sources of these compounds.[48] Within months of each other, two laboratories reported the isolation of endophytes capable of producing podophyllotoxin and related analogues.[48,49] Puri isolated *Trametes hirsuta* from the dried rhizomes of *Podophyllum hexandrum* collected from the north-western Himalayan region of Jammu & Kashmir, India,[48] while Porter and colleagues isolated two different strains of *Phialocephala fortinii* from rhizomes of *Podophyllum peltatum.*[49]



podophyllotoxin **46**

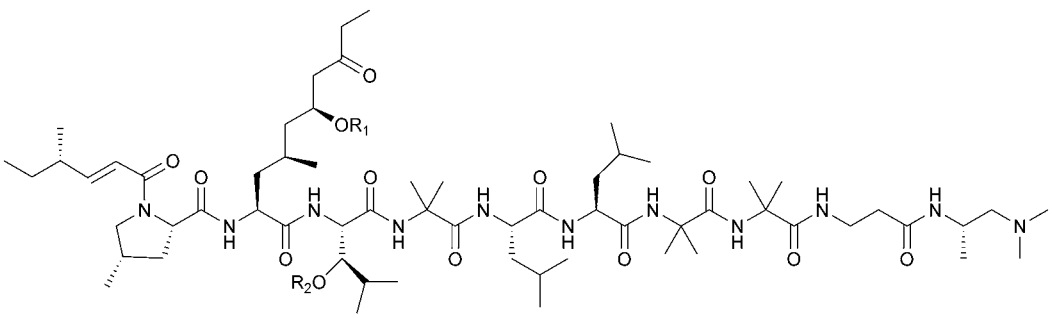
3.3.12 Peptides. Leucinostatin A **47** was isolated almost forty years ago from cultures of *Penicillium lilacum*.[92] It has received much attention over the years because of its potent biological activity against several different cell lines. Scientists have found that **47** inhibits prostate cancer growth through the reduction of insulin-like growth factor-I expression in prostate stromal cells.[93] *Acremonium* sp. isolated from *Taxus baccata* was also shown to produce **47** when grown in liquid culture.[94] The fungal endophyte also produced leucinostatin A di-*O*-β-glucoside **48**, a glycosylated analogue of **47** which had an LD_{50} of >25 nM against breast cancer cell line BT-20, compared to leucinostatin A, which had an LD_{50} of 2 nM.[94]

3.3.13 Polyketides. The novel oblongolides Y **49** and Z **50** were isolated from *Phomopsis* sp. BCC 9789 associated with *Musa acuminata* (wild banana).[95] Oblongolide Y showed cytotoxic activity against human breast cancer cell line BC with an IC_{50} value of 48 μM while oblongolide Z exhibited cytotoxicity against KB (human oral epidermoid cancer), BC and NCI-H187 (small-cell lung cancer), and non-malignant (Vero) cell lines with IC_{50} values of 37, 26, 32 and 60 μM, compared to doxorubicin as a positive control, which had IC_{50} values of 0.24 μM (KB), 0.30 μM (BC) and 0.08 μM (NCI-H187).[95]

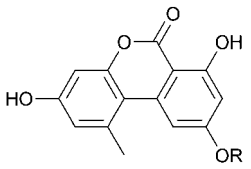


R = CH_3 oblongolide Y **49**
R = CH_2CH_2Ph oblongolide Z **50**

An endophytic *Alternaria* sp., isolated from the Egyptian medicinal plant *Polygonum senegalense*, produced several tricyclic lactone polyketides including the known alternariol **51**, alternariol 5-*O*-sulfate **52** and alternariol 5-*O*-methyl ether **53**. Compound **52** has not been previously reported.[96] These compounds were cytotoxic to L5178Y mouse lymphoma cells with EC_{50} values of 1.7, 4.5 and 7.8 μg/mL respectively, compared to the positive control kahalalide F, which had an EC_{50} value of 6.3 μg/mL. Structure–activity studies suggest that the free hydroxyl group at C-4′ plays an important role in mediating cytotoxicity, because substitution of this functional group decreased the activity significantly in other compounds isolated from the same endophyte.[96]

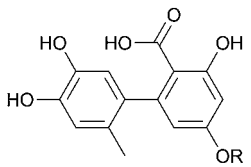


$R_1 = R_2 = H$ leucinostatin A **47**
$R_1 = R_2 =$ glucose leucinostatin A di-glycoside **48**



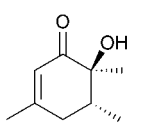
R = H alternariol **51**
R = SO_3H alternariol 5-*O*-sulfate **52**
R = CH_3 alternariol 5-*O*-methyl ether **53**

The same fungal endophyte also produced two bicyclic acid derivatives – the known altenusin **54** and the novel desmethylaltenusin **55**.[96] These compounds also exhibited significant cytotoxic activity against L5178Y cells, with EC_{50} values of 6.8 and 6.2 μg/mL respectively, compared to the positive control kahalalide F.[96]

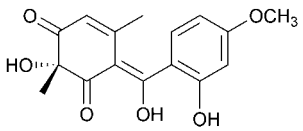


R = CH_3 altenusin **54**
R = H desmethylaltenusin **55**

Leptosphaerone C **56** and penicillenone **57** are novel polyketides isolated from *Penicillium* sp. JP-1, an endophytic fungus associated with the mangrove plant *Aegiceras corniculatum*.[29] Leptosphaerone C showed activity against A549 cells (adenocarcinomic human alveolar basal epithelial) with an IC_{50} value of 1.45 μM, and penicillenone **57** exhibited cytotoxicity against P388 leukemia cells with an IC_{50} value of 1.38 μM.[29]

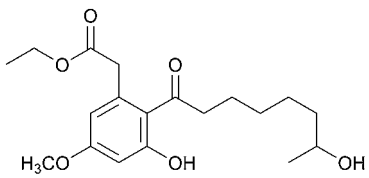


leptosphaerone C **56**



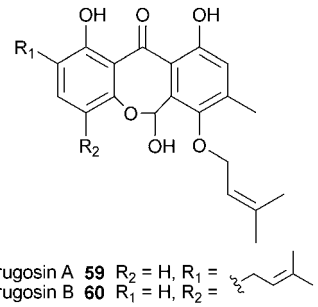
penicillenone **57**

Another mangrove endophyte *Phomopsis* sp. ZSU-H76 was the source of 2-(7′-hydroxyoxooctyl)-3-hydroxy-5-methoxybenzene acetic acid ethyl ester **58**, a new polyketide. The endophyte was isolated from isolated from the stem of *Excoecaria agallocha* from Dong Zai, Hainan, China.[97] Compound **58** exhibited cytotoxicity towards HEp-2 and HepG2 cell lines, with IC_{50} values of 25 and 30 μg/mL.[97]



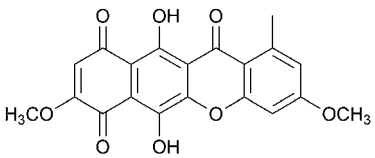
2-(7′-hydroxyoxooctyl)-3-hydroxy-5-methoxybenzene acetic acid ethyl ester **58**

Arugosins A **59** and B **60** are benzophenone polyketides isolated from *Emericella nidulans* var. *acristata*, an endophyte of a Mediterranean green alga.[31] Both compounds showed moderate antitumor activity against 7 out of 36 human tumor cell lines at a concentration of 10 μg/mL.[31] The reference compound adriamycin, tested in parallel in the same assays, was more potent, with an IC_{50} value of 0.016 μg/mL.



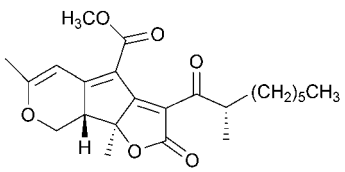
arugosin A **59** R_2 = H, R_1 =
arugosin B **60** R_1 = H, R_2 =

Bikaverin **61** a polyketide isolated from *Fusarium oxysporum* strain CECIS, an endophyte of *Cylindropuntia echinocarpus*, exhibited cytotoxicity against a panel of four sentinel cancer cell lines, NCI-H460 (non-small-cell lung), MIA Pa Ca-2 (pancreatic), MCF-7 (breast), and SF-268 (CNS glioma) with IC_{50} values of 0.43, 0.26, 0.42 and 0.38 μM, respectively. It was compared to the standard compound doxorubicin, which exhibited IC_{50} values of 0.01, 0.05, 0.07 and 0.04 μM respectively.[73]

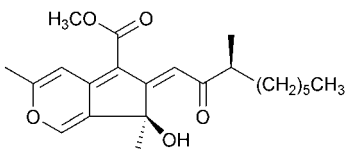


bikaverin **61**

Two novel polyketides, sequoiatone A **62** and B **63**, were isolated from the endophyte *Aspergillus parasiticus* from the bark of *Sequoia sempervirens*.[98] The compounds showed moderate and somewhat selective inhibition of human tumor cells, with greatest efficacy against breast cancer cell lines. Most of the GI_{50} values were between 4–10 μM, with LC_{50} values >100 μM.[98]



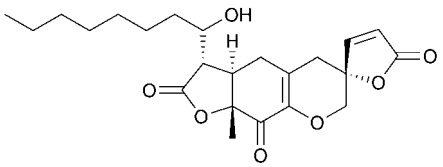
sequoiatone A **62**



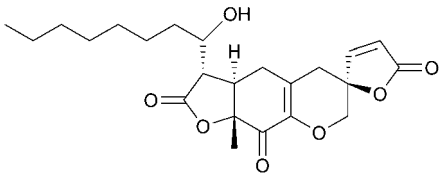
sequoiatone B **63**

The same endophyte yielded sequoiamonascins A **64** and B **65**, which exhibited cytotoxic activity against MCF7 (breast), NCI-H460 (lung), and SF-268 (CNS) when tested by NCI in their human cell line screen.[99] The NCI Drug Therapeutic Program reported the activity in terms of percent of growth of treated cells compared to untreated cells; values below 32% were considered active. At concentrations of 10 μM, **64** allowed 1%, 1% and 2%, (percent of growth) respectively for each treated cell type, while **65** allowed 19%, 4% and 15% (percent of growth) of treated

cancer cells respectively.[99] In the 60-human cell line assay, **64** had a median log GI_{50} of −5.00, below the potency threshold established by NCI to warrant further study. Compound **64** showed selective activity towards all six leukemia cell lines, one breast cancer cell line, and two melanoma cell lines, with median log GI_{50} values approaching −6.00.[99]

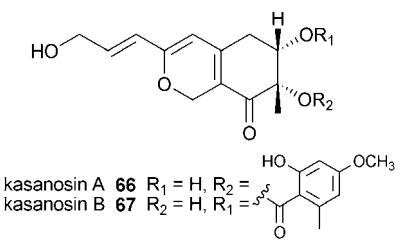


sequoiamonascin A **64**



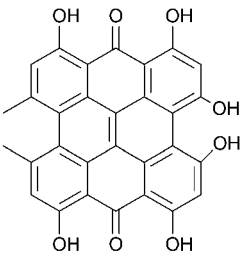
sequoiamonascin B **65**

Kasanosins A **66** and B **67** are novel azaphilones isolated from cultures of *Talaromyces* sp. derived from seaweed.[100] These compounds were not evaluated for cytotoxicity against specific cancer cell lines. Instead, the authors focused on the ability of these compounds to selectively inhibit specific DNA polymerases. Compounds **66** and **67** specifically inhibited eukaryotic polymerases β and γ. Compound **66** was more potent than **67**, with IC_{50} values of 27.3 (DNA pol β) and 35.0 μM (DNA pol γ). DNA polymerases are important target molecules of antitumor agents, especially for antimetabolite nucleosides that include 1-β-D-arabinofuranosylcytosine (araC) and 2′-deoxy-2′,2′-difluorocytidine (gemcitabine).[101] There are several subtypes of mammalian DNA polymerases, and their localization and function have been clarified. DNA polymerases α, δ and ε appear to be responsible for DNA replication, whereas DNA polymerases β, δ and ε appear to work in DNA repair.[101] DNA polymerase γ is encoded in the nucleus but localizes in the mitochondria, and is responsible for mitochondrial DNA replication.[101] Compounds **66** and **67** have very high specificity for families of DNA polymerases, which might be useful in the development of a drug design strategy for immunosuppressive and/or anti-cancer chemotherapy agents.[100]

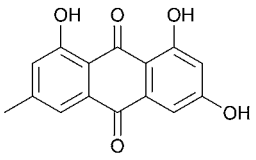


kasanosin A **66** R_1 = H, R_2 =
kasanosin B **67** R_2 = H, R_1 =

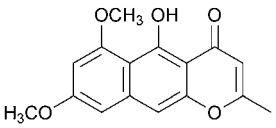
Hypericin **68**, a naphthodianthrone derivative, is a plant-derived metabolite with a long history of medicinal use. It was originally isolated from the herb *Hypericum perforatum* (St. John's Wort) which has been used since ancient times to treat depression and other ailments. [102,103] Hypericin has been reported to be a very active component of the plant, and in various studies has been found to be a potent MAO inhibitor[103] as well as a potent antiviral against a plethora of enveloped viruses including HIV-1, HSV-1, HSV-2, BVDV, BIV, and influenza A.[104] Several *in vitro* studies have revealed the multifaceted cytotoxic activity of **68** as a result of photodynamic activity.[105–107]



hypericin **68**



emodin **69**

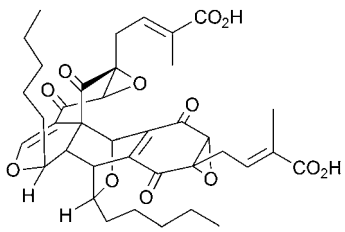


rubrofusarin B **70**

For the first time hypericin **68**, along with emodin **69**, was isolated from a stem endophyte of *H. perforatum* harvested in India. The fungus was code-named INFU/Hp/KF/34B, and extensive analysis suggested it was related to *Chaetomium globosum*.[108] The organism was ultimately identified as *Thielavia subthermophila*.[109] The fungal extract containing compounds **68** and **69** exhibited photodynamic cytotoxicity against the human acute monocytic leukemia cell line (THP-1) in two different assays. THP-1 cells were exposed to varying concentrations of the fungal extract in the dark and after the extract had been irradiated with visible light for 20 minutes. In the resazurin-based assay, dark vs. light cell viability was 92.7 vs. 4.9%, and in the ATPlite assay, dark vs. light cell viability was 91.1 vs 1.0%.[109]

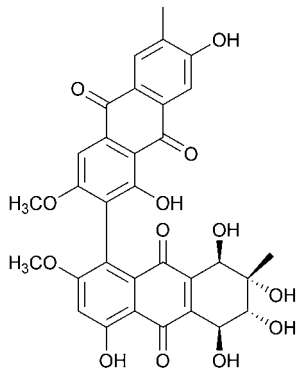
The known naphtha-γ-pyrone rubrofusarin B **70** was isolated from *Aspergillus niger* IFB-E003, an endophyte of *Cynodon dactylon*. It was cytotoxic to colon cancer cell line SW1116, with an IC_{50} value of 4.5 μg/mL, compared to the positive control 5-fluorouracil at 5 μg/mL.[110] Rubrofusarin B also reversed multidrug resistance of human epidermal KB carcinoma cells.[110]

3.3.14 Quinones. Torreyanic acid **71** is an unusual dimeric quinone isolated from *Pestalotiopsis microspora*, an endophyte of *Torreya taxifolia*.[111] In general, torreyanic acid was found to be 5–10 times more potent against cell lines that are sensitive to protein kinase C (PKC) agonists, and it was suggested that **71** causes cell death by apoptosis. IC_{50} values for **71** were between 3.5 μg/mL for human colorectal neuroendocrine cell carcinoma (NEC) to 45 μg/mL for human adenocarcinomic alveolar basal epithelial cells (A549), with a mean value of 9.4 μg/mL for 25 different cell lines. Torreyanic acid also showed G1 arrest of G0 synchronized cells at the 1–5 μg/mL level depending on the cell line.[111]

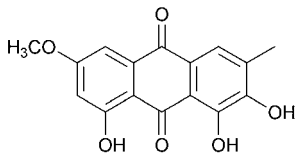


torreyanic acid A **71**

Five novel and eight known compounds were isolated from *Stemphylium globuliferum*, an endophyte of the Egyptian medicinal plant *Mentha pulegium*.[112] Each of the compounds isolated from this fungus was tested for cytotoxicity against L5178Y mouse lymphoma cells. Of the five novel compounds an unresolved mixture of alterporriol G and its atropisomer alterporriol H **72** exhibited the most potent cytotoxicity, with an EC_{50} value of 2.7 μg/mL. The previously reported compound 6-*O*-methylalaternin **73** also exhibited potent cytotoxicity, with an EC_{50} value of 4.2 μg/mL. Kahalalide F was tested as a positive control and exhibited an EC_{50} value of 6.3 μg/mL. The compounds were also tested for kinase inhibitory activity in an assay involving 24 different kinases. The atropisomers **72** and compound **73** were the most potent kinase inhibitors, displaying EC_{50} values between 0.64 and 1.4 μg/mL towards individual kinases.[112] The authors suggested that the inhibition of protein kinases could be the basis of the observed cytotoxic activity.[112]

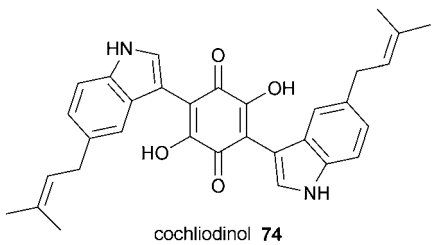


alterporriol G and H (atropisomers) **72**

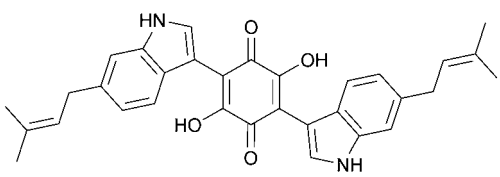


6-*O*-methylalaternin **73**

An endophytic *Chaetomium* sp. was isolated from the stem of *Salvia officinalis*.[113] Compounds were tested for cytotoxicity against L5178Y mouse lymphoma cells. Two compounds were isolated, the previously reported cochliodinol **74** and isocochliodinol **75**.[113,114] Compound **74** was an order of magnitude more potent than its isomer, with an EC_{50} of 7.0 μg/mL, compared to 71.5 μg/mL for compound **75**.[113]

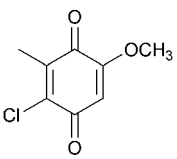


cochliodinol **74**

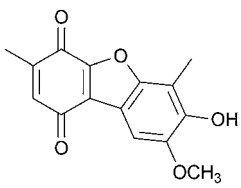


isocochliodinol **75**

Two novel benzoquinone derivatives, 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione **76** and xylariaquinone A **77**, were isolated from *Xylaria* sp., an endophytic fungus of *Sandoricum koetjape*. Compound **76** showed potent cytotoxicity against African green monkey kidney fibroblasts (Vero cells) with an IC_{50} value of 1.35 μM compared to the positive control ellipticine, with an IC_{50} value of 2.03 μM.[115] Vero cells are non-malignant, but some researchers have speculated that certain host cells actually provide the necessary 'fertile soil' for cancer progression.[116,117] These cells include endothelial cells and pericytes forming blood and lymph vessels attracted to the cancer cells by vascular endothelial growth factor (VEGF); nerve cells; fibroblasts which can be converted into myofibroblasts by cancer cell released transforming growth factor (TGF)-β; inflammatory cells which can be attracted by cancer chemokines and osteoclasts activated by metastatic cancer cells in the bone marrow. All these host cells engage in continuous molecular cross talk with the cancer cells, influencing invasion and metastasis. Tumor-associated host cells are themselves invasive, and some of them arrive at the site of metastasis ahead of the cancer cells.[116] These cancer-associated functions have led some researchers to consider these host cells to be appropriate targets for chemotherapy.

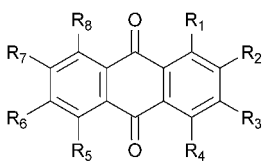


2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione **76**



xylariaquinone A **77**

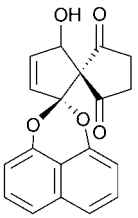
Fourteen previously reported anthracenedione derivatives were isolated from *Halorosellinia* sp. (no. 1403) and *Guignardia* sp. (no. 4382), fungal endophytes of an unspecified mangrove plant.[118] All fourteen compounds exhibited some degree of cytotoxicity, but six compounds exhibited the greatest potency. These compounds, anthracenedione derivatives **78–83**, exhibited cytotoxicity towards KB and KBv200 cell lines, with IC_{50} values between 3.7 and 70 μM. Compound **80** was the most potent, with an IC_{50} value of 3.17 μM (KB) and 3.21 (KBv200). Compounds **78**, **79** and **82** also exhibited cytotoxicity against KBv200, with IC_{50} values between 3.21 and 91 μM. The literature suggests that both the number and location of hydroxyl groups play a key role in cytotoxicity. Compound **80** has a single hydroxyl group and is the most potent cytotoxic agent against both cell lines.[118]



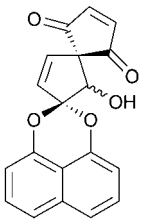
anthracenedione derivative 1 $R_1 = R_3 = OCH_3$, $R_6 = CH_3$ **78**
anthracenedione derivative 5 $R_1 = R_4 = OH$, $R_7 = CH_3$ **79**
anthracenedione derivative 6 $R_1 = OH$, $R_3 = CH_3$ **80**
anthracenedione derivative 7 $R_1 = R_8 = OH$ **81**
anthracenedione derivative 9 $R_1 = R_3 = OH$, $R_6 = OCH_3$, $R_8 = CH_3$ **82**
anthracenedione derivative 14 $R_1 = OH$, $R_2 = R_4 = OCH_3$, $R_7 = CH_3$ **83**
undefined R = H

3.3.15 Spirobisnaphthalenes. The spirobisnaphthalenes are a relatively new class of compounds that was first reported in 1990. They possess two naphthalene-derived C_{10} units bridged through a spiroketal linkage. Spiro-mamakone A **84** was isolated

from an unspecified nonsporulating endophytic fungus (Mycelia sterilia) isolated from the native New Zealand tree *Knightia excelsa* (rewarewa).[119] Compound **84** exhibited potent cytotoxic activity against P388 (murine leukemia cell line), with an IC_{50} value of 0.33 μM. The compound also exhibited potent antimicrobial activity.[119]



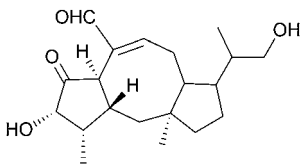
spiromamakone A **84**



spiropreussione A **85**

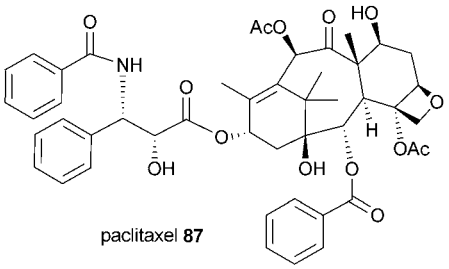
The endophytic fungus *Preussia* sp. was isolated from a mature stem of *Aquilaria sinensis* (Thymelaeaceae), collected from Guangxi Medicinal Arboretum.[120] It produced a series of novel spirobisnaphthalenes, one of which, spiropreussione A **85**, exhibited *in vitro* cytotoxicity against the A2780 human ovarian carcinoma cell line and the BEL-7404 human liver carcinoma cell line, with IC_{50} values of 2.4 and 3.0 μM, respectively. Compound **85** was inactive (IC_{50} >10 μM) against the HCT-8 (colon carcinoma), BGC-823 (gastric carcinoma), and A549 (lung adenocarcinoma) human cancer cell lines. None of the other novel compounds exhibited cytotoxicity in these assays at the concentrations tested.[120]

3.3.16 Diterpenes. Periconicin B **86** is a fusicoccane diterpene isolated from the endophytic fungus *Periconia atropurpurea*, associated with *Xylopia aromatica*.[121] Compound **86** exhibited potent cytotoxic activity against the two mammalian cell lines, HeLa (cervical cancer) and CHO (Chinese hamster ovary). It decreased cell viability of HeLa cells and CHO cells with an IC_{50} of 8.0 μM, showing potency similar to that of cisplatin, a well known antineoplastic agent (IC_{50} 5.0 μM) used as a cytotoxic positive control.[121]



periconicin B **86**

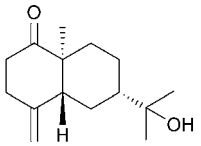
It could be reasonably argued that no other secondary metabolite has had such a dramatic effect on cancer chemotherapy as Taxol® (paclitaxel) **87**.[9,122] This highly functionalized diterpene is the prototypical taxane, isolated from the bark of the Northwest Pacific yew tree *Taxus brevifolia* for the first time by Wani *et al.* in 1971.[7,123] (When it was developed commercially by Bristol-Myers Squibb, the generic name was changed to 'paclitaxel' and Taxol® was trademarked.) Paclitaxel showed early promise against a series of human solid tumor xenografts in nude mice including CX-1 colon and MX-1 breast xenografts.[122] These early results were encouraging. A real turning point in the paclitaxel saga, however, was the discovery of its unique activity as a promoter of tubulin polymerization.[124] Although other clinically useful drugs were known to act as anti-mitotic agents and inhibitors of tubulin polymerization, paclitaxel was the first compound to exhibit the opposite effect on tubulin – stabilization of its polymer.[9,122,124]



paclitaxel **87**

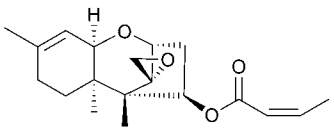
Unfortunately, as paclitaxel garnered more attention for its unique mode of action and potential as a chemotherapeutic agent, it also gained attention because of problems associated with the supply issue. Early estimates suggested that the population of Northwest Pacific yew trees could not adequately supply the projected demands for paclitaxel. Alternative sources were considered for the compound including total synthesis, semi-synthesis and tissue culture.[122] Stierle *et al.* took another approach, and in 1993 reported the isolation of a fungal endophyte from the needles of *T. brevifolia* that produced paclitaxel independently of the tree.[12] The fungus had not been previously described, and was designated *Taxomyces andreanae* in honor of its discoverer.[12] Stierle later reported the discovery of paclitaxel by a second fungus, *Penicillium raistrickii* isolated from the inner bark of a yew tree.[125] Several other scientists have since reported the isolation of paclitaxel from different endophytic fungi associated not only with *Taxus* sp. but with other host plants as well. Strobel reported the production of paclitaxel from *Pestalotiopsis microspora* isolated from *Taxus wallichiana*[126] and a second isolate of *Pestalotiopsis microspora* from bald cypress, *Taxodium distichum*.[127] It has been reported from *P. pausiceta* isolated from *Cardiospermum helicacabum*[128] and from *Pestalotiopsis terminaliae*, an endophytic fungus of *Terminalia arjuna*.[129] It has also been reported from *Chaetomella raphigera*, a second endophytic paclitaxel-producer reported from *Terminalia arjuna*.[130] The same scientists also reported the production of paclitaxel by *Bartalinia robillardoides*, an endophyte of *Aegle marmelos*.[131] This is not a comprehensive list of paclitaxel-producing endophytes, and more producers are reported every year. Fungal paclitaxel has been tested by apoptotic assay against a number of different cancer cell lines, including BT220, H116, HLK210, HL251 and INT-407. As the paclitaxel concentration increased from 0.005–0.05 μM, paclitaxel-induced cell death through apoptosis increased accordingly, but the level of cell death only increased slightly with a further increase to 0.5 μM, while a further increase to 5 μM resulted in a dramatic decrease in cell death.[129]

3.3.17 Sesquiterpenes. A new eudesmane sesquiterpene, *ent*-4(15)-eudesmen-11-ol-1-one **88**, was isolated from the endophytic fungus *Eutypella* sp. BCC 13199 associated with *Etlingera littoralis* (Earth ginger).[89] It showed weak cytotoxic activity against human cancer cells NCI-H187, MCF-7, KB and Vero, with IC_{50} values of 11, 20, 32 and 32 μM respectively.[89]

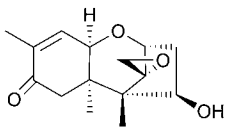


ent-4(15)-eudesmen-11-ol-1-one **88**

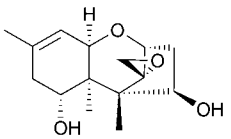
Four cytotoxic sesquiterpene compounds, 8-deoxytrichothecin **89**, trichothecolone **90**, 7α-hydroxytrichodermol **91** and 7α-hydroxyscirpene **92**, were isolated from fungal isolate KLAR 5, which the authors identified as a "sister taxon of *Acremonium crotociniginum*" a mitosporic Hypocreales found in a healthy twig of the Thai medicinal plant *Knema laurina*.[84] Compounds **89** and **91** exhibited selective activity against BC-1 (human breast cancer cells), with effective IC_{50} values of 0.88 and 2.37 respectively, and against NCI-H187 (human small-cell lung cancer cells) with IC_{50} values of 1.48 and 1.73 μM repsectively, compared to the standard drug elipticine that exhibited an IC_{50} value of 0.63 μM against the BC-1 cell line. These compounds were not active against the KB cell line (human epidermoid cancer of the mouth).[84] Compounds **90** and **92** were moderately active against all three cancer cell lines with IC_{50} values of 10.06, 11.31, 12.90 μM and 21.53, 27.76, 8.47 μM repectively.[84]



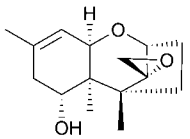
8-deoxytrichothecin **89**



trichothecolone **90**

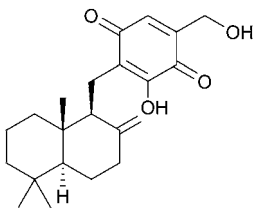


7α-hydroxytrichodermol **91**



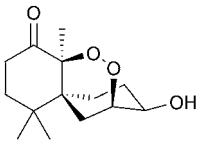
7α-hydroxyscirpene **92**

Phyllosticta spinarum was isolated from *Platycladus orientalis* a plant of the Sonoran desert.[132] Although the fungus produced a series of compounds, only tauranin **93** exhibited cytotoxic activity against several cancer cell lines: NCI-H460 (non small cell lung cancer), MCF-7 (breast cancer), SF-268 (CNS cancer – glioma), PC-3M (metastatic prostate cancer) MIA Pa Ca-2 (pancreatic carcinoma) at values of 4.3, 1.5, 1.8, 3.5, and 2.8 μM, respectively.[132]

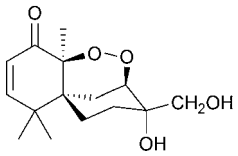


tauranin **93**

Merulin A **94** (nor-chamigrane endoperoxide) and merulin C **95** (chamigrane endoperoxide) are two new sesquiterpenes produced by the endophytic fungus XG8D, a basidiomycete isolated from the mangrove plant *Xylocarpus granatum* (Meliaceae). Both compounds exhibited significant cytotoxicity against human breast cancer (BT474) and colon cancer (SW620) cell lines with IC_{50} values of 4.98 and 1.57 μg/mL for BT474, and 4.84 and 4.11 μg/mL for SW620, respectively compared to doxorubicin used as a positive control with IC_{50} values of 0.53 and 0.09 μg/mL against BT474 and SW620 cell lines, respectively.[133]

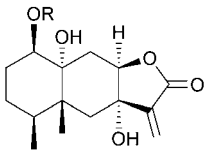


merulin A **94**

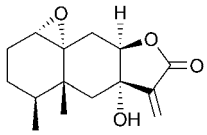


merulin C **95**

Three novel eremophilane-type sesquiterpenes were isolated from the endophyte *Xylaria* sp. BCC 21097 associated with *Licuala spinosa*.[134] The three compounds, eremophilanolide 1 (**96**), 2 (**97**) and 3 (**98**) exhibited moderate cytotoxic activity with IC_{50} values of 3.8–21 μM against cancer cell lines KB, MCF-7, and NCI-H187.[134]

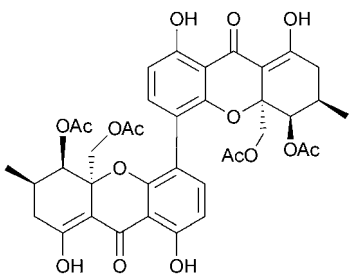


R = H eremophilanolide 1 **96**
R = CH_3 eremophilanolide 2 **97**

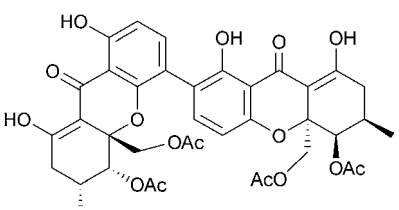


eremophilanolide 3 **98**

3.3.18 Xanthones. Phomoxanthones A **99** and B **100**, two novel xanthone dimers, were isolated from the fungus *Phomopsis* sp. BCC 1323, an endophyte of *Tectona grandis*. Both compounds exhibited impressive cytotoxic activity against KB cells, BC-1 cells and non-malignant Vero cells. Phomoxanthone A **99** had IC_{50} values of 0.99, 0.51 and 1.4 μg/mL, respectively while Phomoxanthones B **100** had IC_{50} values of 4.1, 0.70 and 1.8 μg/mL, compared to the standard compound ellipticine, which had IC_{50} values of 0.46 μg/mL for KB cells and 0.60 μg/mL for BC-1 cells respectively.[135]



phomoxanthone A **99**



phomoxanthone B **100**

4 Conclusion

This review highlights the importance of endophytic fungi – those hidden, subtle inhabitants of the interstitial spaces in plants – as a source of secondary metabolites with promising anticancer activity. The search for new anticancer agents and for new sources of potent plant-derived compounds is critical, considering the number of deaths associated with cancers on an annual basis, and the likelihood that this number will increase in the future. Access to a limited number of cancer chemotherapies, their serious side effects and high cost make treatment particularly challenging. In addition, many therapies do not effectively treat certain cancers, and multi-drug-resistant tumors exacerbate treatment complexity. The discovery of new chemotherapeutic agents is therefore a key goal for natural product and medicinal chemists.

Endophytic fungi are proving to be prolific producers of anti-cancer compounds from many different chemical classes. In the past ten years, 100 compounds with significant cytotoxicity were reported from endophytic fungi, and the isolation of anticancer compounds has been increasing over five year intervals – it is interesting to note from 1990–1995, only a single novel anticancer agent was reported from endophytic fungi. The most exciting discovery in this area of research during this period was a fungus capable of producing paclitaxel, a new anticancer agent associated with a higher plant host, the Northwest Pacific yew tree. This discovery spurred interest not only in fungal endophytes as a source of novel anticancer agents, but also in endophytes as an alternative source of valuable higher-plant metabolites.

In the next five-year interval, 1996–2000, only two novel compounds were discovered, but a fungal source of vincristine was reported. From 2001–2005, nine novel anticancer agents were reported, as well as the first fungal source of the important anticancer agent camptothecin. From 2006–2010, 75 compounds with significant cytotoxicity were reported. Of these, 43 were novel structures. During this same period fungal sources of the plant-derived anticancer compounds podophyllotoxin and hypericin were discovered, as well as new fungal sources of camptothecin and paclitaxel.

A fungal source of a desired anticancer agent is of particular value, as fungal fermentation provides a virtually inexhaustible source of desired metabolites. As natural products chemists turn their attention to endophytic fungi, the number of new compounds isolated should increase over the next five years. Many of the compounds discussed in this review had IC_{50} values comparable to those of the standard reference drugs, making the search for anticancer compounds isolated from endophytic fungi a promising one. Novel compounds or previously isolated compounds are readily available and accessible to whatever specific anticancer screens researchers use for isolation and evaluation. As our understanding of the mechanisms associated with the onset and metastasis of cancers increases, our ability to use this knowledge to select for ever more potent and selective compounds should increase commensurately. Endophytic fungi will continue to provide a fertile arena for these quests.

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