doi.org/10.1002/cbdv.202100976

REVIEW

CHEMISTRY & BIODIVERSITY

Exploration of Potent Cytotoxic Molecules from Fungi in Recent Past to Discover Plausible Anticancer Scaffolds

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Fungi are known to produce diverse scaffolds possessing unique biological activities, however, to date, no molecule discovered from a fungal source has reached the market as an anti-cancer drug. Every year number of cytotoxic molecules of fungal origin are getting published and critical analysis of those compounds is necessary to identify the potent ones. A review mentioning the best cytotoxic fungal metabolites and their status in the drug development was published in 2014. In this report, we have included 176 cytotoxic molecules isolated from fungi after 2014 and categorized them according to their potencies such as IC_{50} values below 1 μM, 1–5 μM, and 5–10 μM. The emphasis was given to those 42 molecules which have shown IC_{50} less than 1 μM and discussed to a great extent. This review shall provide potent scaffolds of fungal origin which can be given priority in the development as a drug candidate for cancer therapeutics.

Keywords: potent fungal metabolites, cancer drug discovery, endolichenic fungi, endophytic fungi, terrestrial and marine fungi.

Introduction

Scientists are zealously trying to reduce the toxicity of the current chemotherapeutics by coalescing them with nature based molecules.[1,2,3] Fungi, the largest eukaryotic kingdom can produce compounds having immeasurable structural diversity belonging to classes azaphilones, cytochalasans, macrolides, anthracenones, naphthalenones, and many more.[4] A list of molecules isolated from fungi include hypothemycin, radicicol, demethoxyviridin, fussicocin A, destruxin B, fumagillin, cytochalasin E etc.[5] Although, the focus of fungal metabolites have been limited towards the antimicrobial activity, fungi have immense potential to produce cytotoxic secondary metabolites in response to predators, UV radiation, along with competition from other microbes.[6,7] The polyketide synthase pathway in fungi is known to produce diverse scaffolds with antitumor activity.[8]

Despite the significant contribution, no molecule has yet reached the market as an anti-cancer drug having a fungal origin.[9] This indicates the existence of lacunas in the process of converting the potent molecules from fungi into a suitable drug candidate. A cytotoxic drug candidate must at least display (i) selectivity between cancer cells and normal cells (ii) active against multidrug-resistant (MDR) cancer cells; and (iii) should have a specific mechanism of action.[4] Further, the selected leads have to go through rigorous drug development processes before it reaches the patients. To invigorate the research interest for fungal secondary metabolites, it is important to re-analyse potent cytotoxic molecules reported from fungi to understand its current standing in process of drug discovery and development. This

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Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202100976

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Pranali S. Pradhan M.S (Pharm) in Natural Products from National Institute of Pharmaceutical Education and Research, Ahmedabad (2021) with thesis titled as 'Isolation and Characterization of Bioactive Molecules Selected through LC/MS Based Dereplication from Endolichenic Fungi', She has completed graduation in pharmacy from Institute of Pharmaceutical Education & Research, Maharashtra, India. She is currently a junior research scientist in Phytolife Sciences Pvt. Ltd., Halol, Gujarat. Her area of research includes bioactivity guided isolation, structural elucidation of novel compounds, standardization of various products and technology transfer development for large scale isolation of active phytoconstituents.

Ashwini Armarkar completed her M.S (Pharm) in Natural Products in 2019 at National Institute of Pharmaceutical Education & Research, Ahmedabad, Gujarat. She has one-year industry experience at Phytolife Sciences Pvt. Ltd., Halol, Gujarat. She is currently working as Assistant Professor in Department of Pharmacognosy at Datta Meghe College of Pharmacy Wardha, Maharashtra. She is working on research project entitled as 'Phytochemical screening of Porona Paniculata Roxb. extract and evaluation of its Acute toxicity in Albino Wistar Rats', Her research interests include isolation of bioactive molecules from natural origin and associated analytical method development towards drug discovery program.

Komal Pandey graduated from the Indian Institute of Technology-Banaras Hindu University (IIT-BHU) with a master's degree in pharmacy from the Department of Pharmaceutical Engineering and Technology. She is currently doing her PhD at the National Institute of Pharmaceutical Education and Research-Ahmedabad, where she is supervised by Dr. Abhijeet Kate. Her study focuses on isolating novel bioactive chemicals from endophytic fungi and plants with 'galls'.

Prof. Kiran Kalia has served as founder director of National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad. She received her Ph.D. degree in 1984 from CSIR-IITR, Lucknow. Her research interest include diabetic nephropathy, oral cancer, prognostic biomarkers and new generation drug delivery systems. She was actively involved in teaching masters, doctoral students having 36 years of teaching and 42 years of research experience at SPU and was the visiting Professor at School of Health Sciences, Purdue University, USA in 2006. She has 187 publications in various national and international peer-reviewed journals with 3810 citation, h-index-32 and i10index-76, filed 17 Indian patents and delivered 75 plenary lectures/lead lectures/ presentations at various national and international platforms at Malaysia, Singapore, Czech Republic, France, China and USA. She has guided 24 PhDs and over 57 M.Sc. dissertation students. She has received extramural research grants to complete 20 major research projects.

Prof. Priyani Paranagama is the Chair of Chemistry, University of Kelaniya and one of the past Presidents of Institute of Chemistry Ceylon (successor to the Chemical Society of Sri Lanka). She obtained her M.Phil and Ph.D. degrees from University of Kelaniya in 1990 and University of Glasgow in 1994 respectively in Bio Organic Chemistry. She also served as the Dean in 2016 and the President of Institute of Chemistry Ceylon in 2019/2020. She is a member of Executive Board of Commonwealth Chemistry. Currently she served as the Director, Institute of Indigenous Medicine, University of Colombo. She is a fellow of Institute of Chemistry and a Chartered Chemist (CChem), Royal Society of Chemistry. She is the Chairperson of the Women Chemist's committee and the Board of Trustees of Institute of Chemistry Ceylon. She was awarded the Dr. C. L. de Silva Gold medal in 2015 and Prof. M. U. S. Sultanbawa gold medal in 2008 for the outstanding achievements in Chemistry. She is a recipient of several national and international research grants and awards. She has published over 61 research articles in indexed journals, written or edited 11 books/book chapters and published over 130 communications. She has successfully supervised over 21 postgraduate students. Her research interests are isolation and characterization of natural products, analysis of spices and essential oils, value addition of natural products and toxicity of trace metals.

Abhijeet S. Kate received Ph.D. degree in Marine Natural Products from Florida Atlantic University, Boca Raton, FL, USA in 2008. He has 13 years of industry experience where he was associated with eminent organizations like Piramal Enterprises Limited and SABIC. Since 2017, he has been with the National Institute of Pharmaceutical Education and Research, Ahmedabad, where he is currently working as an Associate Professor. His research interest includes finding novel scaffolds from marine and terrestrial bacteria, fungi and plants by applying LC-HRMS dereplication strategies. He has optimized downstream processes for large scale isolation of Natural Products and developed a methodology for fingerprinting of extracts by hyphenated techniques in Natural Products based drug discovery program. Abhijeet's research group is investigating lichens, grown on mangroves from Gulf of Kutch and Gulf of Khambhat region of Gujarat, to find bioactive molecules.

information will prove beneficial to pick top molecules and push them forward in the process of drug discovery.

Cytotoxic compounds isolated from fungi from 1964 to 2013 are mentioned in a review published in 2014, with the key focus upon the mechanism of action of 18 molecules out of the total described 51 molecules.[10] Plinabulin NPI-2358 (Phase III) and PX-866, a wortmannin synthetic analog (Phase II) are leading in process of drug discovery while other fungal metabolites like anguidine, aphidicolin, rhizoxin, fumagillin, illudin S, phenylahistin, and their synthetic derivatives have reached up to initial stages of clinical trials.[11,12] This review aims to cover a thorough assessment of fungal secondary metabolites having anti-cancer activity reported from endolichenic fungi, endophytic fungi, marine fungi, and terrestrial fungi from January 2014 to June 2021. Three categories have been created such as potent (IC_{50} below 1 μM) moderately potent (IC_{50} 1–5 μM), and less potent (IC_{50} 5–10 μM). In total, 176 cytotoxic molecules belonging to various classes like alkaloids, terpenoids, polyketides, and macrolide etc., have been represented graphically in *Figure 1*. Endophytic fungi have largely contributed towards the anti-cancer molecules (117) which were followed by marine fungi (39).

The data analysis revealed 42 molecules (enlisted in *Table 1*) have shown potent anti-cancer activity, 80 molecules showed moderate activity and 54 molecules were having IC_{50} between 5–10 μM. The details are given in the supplementary file (*Table S1* and *S2*). These tables contain information like the name of fungi, compound name, cell lines, and IC_{50}. Potent molecules have been segregated into various classes such as penicilazaphilones, alkaloids, ophiobolins, macrolides, terpenoids, azanthraquinone, and miscellaneous. The available information on these molecules such as the mechanism of action, structural activity relationship, scaffold chemistry along with yield and fungal growth conditions have been critically analyzed and included in this review. The search engines like Sci Finder, Google Scholar, PubMed, and PubChem have been used to fetch the data of the discussed molecules.

Penicilazaphilones

Azaphilones are polyketide derivatives with pyrone-quinone structures containing an oxygenated bicyclic core and achiral quaternary center.[47] The biosynthesis of azaphilone in fungi is associated with polyketide and fatty acid synthesis pathways. Penicilazaphilones have been isolated from marine fungus *Penicillium sclerotiorum* M-22 with 19.85 mg/L yield. Here, the culture conditions have been optimized by response

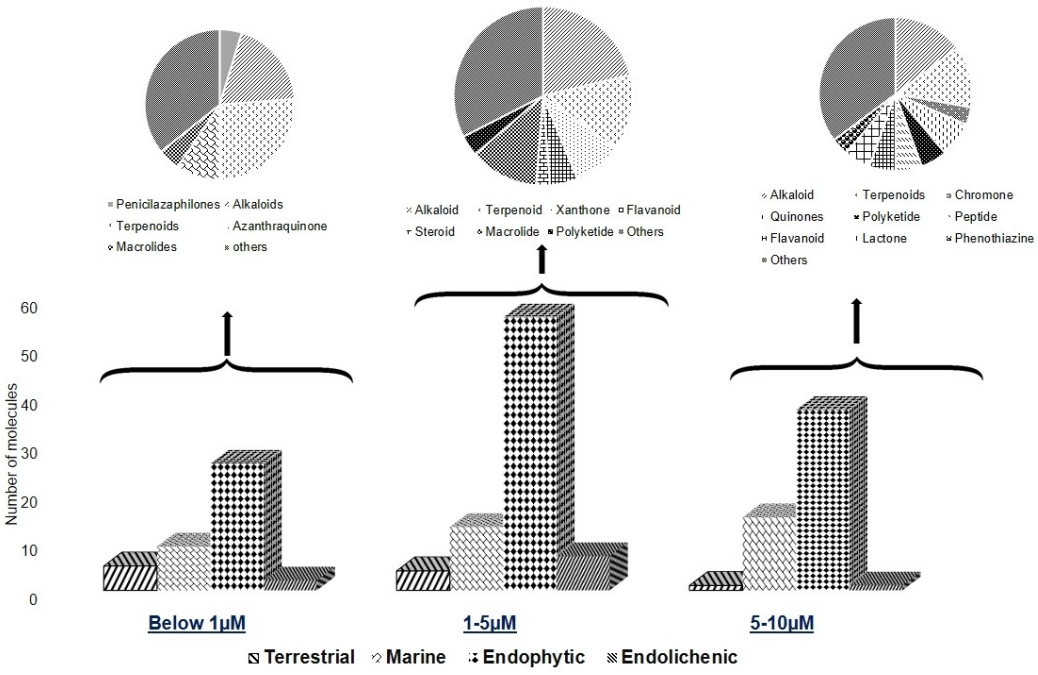


Figure 1. Cytotoxic compounds belonging to different chemical classes and their fungal origin since January 2014–June 2021.

Table 1. Potent cytotoxic compounds isolated from fungi from 2014 onwards with *in vitro* IC_{50} value below 1 μM.

Fungi Source	Compound	*In Vitro* Cell Line Models	IC_{50} Value (μM)	Ref.
MARINE				
Penicillium sclerotiorum	Penicilazaphilones C	Melanoma cells B-16, Human Gastric Cancer Cells SGC-7901	0.06, 0.72	[13]
Chaunopycnis sp. (CMBMF028)	Chaunolidone A	Lung Carcinoma Cell NCI-H460	0.09	[14]
Emericella nidulans	Emestrin B	Colorectal adenocarcinoma WiDr Cells	0.253	[15]
Penicillium sclerotiorum M-22	Penicilazaphilones B	Melanoma cells B-16, Human Gastric Cancer Cells SGC-7901	0.29, 0.44	[13]
Fungus chondrostereum sp.	Chondrosterin J	Human nasopharyngeal cancer cell lines	0.56	[16]
Penicillium brocae MA-231	Brocazines F	NCI-H460	0.89	[17]
Aspergillus flocculosus	14,15-dehydro-6-*epi*-ophiobolin K	HCT-15, NUGC-3, NCI-H23, ACHN, PC-3, MDA-MB-231	0.21, 0.19, 0.18, 0.24, 0.24, 0.14	[18]
Aspergillus flocculosus	14,15-dehydroophiobolin K	HCT-15, NUGC-3, NCI-H23, ACHN, PC-3, MDA-MB-231	0.44, 0.50, 0.61, 0.53, 0.47, 0.63	[18]
Aspergillus flocculosus	14,15-dehydro-6-*epi*-ophiobolin G	HCT-15, NUGC-3	0.96, 0.88	[18]
TERRESTRIAL FUNGI				
Orbiocrella sp. BCC33248	Orbiocrellin B	NCI-H187 Cells	0.7713	[19]
Emericellopsis alkaline	Emericellipsin A	HeLa Tumor Cell Lines	<0.5	[20]
Thermothelomyces thermophiles ATCC 42464	Myceliothermophin A	DLD-1, Hep3B, HGC-27	0.68, 0.93, 0.23	[21]
	Myceliothermophin E	DLD-1, Hep3B, HepG2, HGC-27	0.32, 0.42, 0.26, 0.08	[21]
	Myceliothermophin F	DLD-1, Hep3B, HepG2, HGC-27	0.48, 0.89, 0.80, 0.33	[21]
ENDOLICHENIC FUNGI				
Apiospora montagnei	N-Hydroxyapiosporamide	L5178 Murine Lymphoma Cell	0.2	[22]
Eurotium sp.	Variecolortide B	Caspase-3 Inhibitory Activity	0.8	[23]
ENDOPHYTIC FUNGI				
Chaetomium sp. M336	6-Formamide Chetomin	HeLa,SGC-7901, A549 cell lines	0.006, 0.023, 0.0271	[24]
Penicillium sp. FJ-1	15-Hydroxy-6α,12-epoxy-7β,10αH,11βH-spiroax-4-ene-12-one	MG-63	0.055	[25]
Phomopsis glabrae	PM181110	BXF 1218L, BXF T 24, CXF 269L, CXF HCT 116, CXF RKO, GXF 251L, HNXF CAL 27, LIXF 575L, LXFA 289L, LXFA 526L, LXFA 629L, LXFL 1121L, LXFL 529L, MAXF 401NL, MAXF MDA 231, MEXF 276L, OVXF OVCAR3, PAXF 1657L, PAXF 546L, PAXF PANC 1, PRXF DU 145, PRXF PC3M, PXF 1118L, RXF 486L, SXF TE671, UXF 1138L.	0.046, 0.05, 0.055, 0.048, 0.074, 0.093, 0.039, 0.295, 0.041, 0.021, 0.04, 0.047, 0.05, 0.064, 0.09, 0.09, 0.195, 0.039, 0.016, 0.059, 0.052, 0.047, 0.054, 0.159, 0.082, 0.126,	[26]
Fusarium sp.	Integracide H	KB Cell Lines	0.18	[27]
Fusarium sp.	Integracide F	SKOV-3	0.2335	[27]

CHEMISTRY & BIODIVERSITY

Table 1. (cont.)

Fungi Source	Compound	*In Vitro* Cell Line Models	IC_{50} Value (μM)	Ref.
Fusarium sp.	Integracide G	SKOV-3	0.175	[28]
Aspergillus clavatus L	Aspergillusone D	A549 Cell Line	0.2	[29]
Pseudolagarobasidium acaciicola	7-*epi*-Merulin B	HL-60 Cell Line	0.28	[30]
Penicillium janthinellum HDN13-309	Penicisulfuranols C	HeLa	0.3	[31]
Peyronellaea coffeae-arabicae FT238	11-Dehydroxyepoxyphomalin A	OVCAR3 Cell Line	0.5	[32]
Aspergillus tamarii	Malformin E	MCF-7	0.65	[33]
Phoma sp. YN02-P-3	Phomone D	HL60 Cell Line	0.65	[34]
Pestalotiopsis microspora	7-*O*-Methylnigrosporolide	L5178Y Cell Line	0.7	[35]
Alternaria phragmospora	4-Methoxy-6-methyl-5-(3-oxobutyl)-2H-pyran-2-one	HL-60 Cell Line	0.9	[36]
Phoma macrostoma	Macrophin	Miapaca-2	0.9	[37]
Libertella blepharis	3-*epi*-Waol A	H460 Cell Line	1	[38]
Phialophora mustea	Phialomustins B	T47D Cell Line	1	[39]
Rhytidhysteron rufulum AS21B	Rhytidenone H	Ramos Cell Line, H1975 Cell Line	0.018, 0.252	[40]
Torrubiella sp. BCC 28517	Torrubiellin B	Cal27, HCC38, A2780,	0.3, 0.4, 0.3	[41]
Fusarium chlamydosporium	Fusarithioamide A	BT-549, SKOV-3	0.4, 0.8	[42]
Fusarium chlamydosporium	Fusarithioamide B	BT-549, MCF-7, HCT-116	0.09, 0.21, 0.59	[42]
Penicillium janthinellum HDN13-309	Penicisulfuranols A	HeLa, HL60	0.5, 0.1	[43]
Fusarium solani	7-Desmethyl-6-methylbostrycoidine	HeLa, MDA MB 231, MIA, Paca2	0.71, 0.73, 0.64, 0.34	[44]
Myrthecim roridum A553	Epiroridine acid	SF-268, MCF-7, NCIH 460, Hepg-2 cell lines	0.751, 0.17, 0.36, 0.38,	[45]
Fusarium solani	7-Desmethylscorpinone	HeLa, MIA, Paca2	0.96, 0.98, 0.61	[44]
Aspergillus ustus 094102	21-*epi*-ophiobolin O	A549, HL-60	0.6, 0.8	[46]

surface methodology to achieve this yield. Penicilazaphilones B (**1**) and C (**2**) (*Figure 2*) have shown potent cytotoxicity against melanoma cells B-16 with IC_{50} 0.29 and 0.06 μM, respectively.[13] These molecules have also shown less than 1 μM IC_{50} against human gastric cancer cells SGC7901 and reported to induce dephosphorylation of AKT and block the notch signalling pathway. An *in vivo* experiment using BALB/c-nude mice inoculated with MGC-803 and SGC-7901 cells demonstrated that compound **2** was effective *in vivo* as it significantly suppressed tumor growth (250 mm^3 tumor volume) compared to that in the control group (600 mm^3 tumor volume).[48]

Alkaloids

Disulfide Bridged Alkaloids

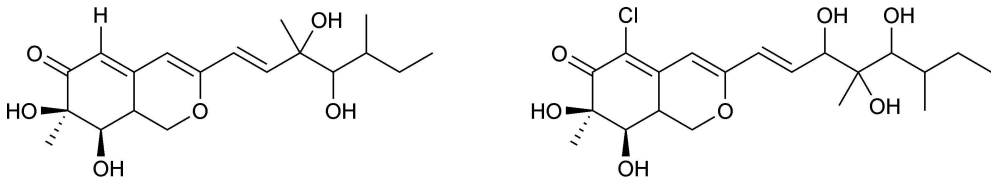
Epipolythiodioxopiperazines (ETP), polysulfide bridged diketopiperazine alkaloids represent a unique class of secondary metabolites produced by fungi with a diketopiperazine core and a sulfur bridge. The first ETP compound gliotoxin was reported in 1936 from *Trichoderma* fungi.[49] Sulfur bridges in the ETP alkaloids are highly reactive, photosensitive, and also a proposed site that contributes to its activity.[50] 6-formamide-chetomin (**3**) is an ETP showed potent cytotoxicity against SGC-7901, HeLa and A549 with IC_{50} 0.023, 0.006, 0.0271 μM, respectively. This compound was isolated from the endophytic fungus *Chaetomium* sp. M336 cultured on potato dextrose agar with percentage extractive yield 0.02% w/w.[24] A large scale production method of these photosensitive ETPs from fungus belonging to genus *Capreolus* has been patented mentioning its fermentation using solid rice medium with purification by column chromatography.[51]

Penicisulfuranols (A–F) were isolated from mangrove endophytic fungus *Penicillium janthinellum* HDN13-309, out of which Penicisulfuranol A (**4**) and C (**5**) (*Figure 3*) showed potent cytotoxic activity through inhibition of C-terminal of Hsp-90 heat shock protein.[52] The percentage extractive yield of (**4**) and (**5**) was 0.25% w/w and 0.23% w/w, respectively.[31] A synthetic method has been developed recently for the production of penicisulfuranols from 2,5-diketopiperazine (DKP) using molybdenum mediated oxidation.[53] Brocazine F (**6**), a bisthiodiketopiperazine derivative is another potent molecule from a marine endophytic fungus, *Penicillium brocae* MA-231 found in the mangrove plant *Avicennia marina*. Six brocazine derivative A-F possessing disulfide bridge were isolated from this fungus and four of them were evaluated for cytotoxicity against different cancer cell lines like Du145, HeLa, HepG2, MCF-7, NCI-H460, SGC-7901, SW1990, SW480, and U251. Among all, brocazine F (**6**) has shown potent antitumor activity at 0.89 μM (IC_{50}) against the NCI-H460. The percentage extractive yield of brocazine F was found to be 0.29% w/w.[17]

Emestrin B (**7**) is also an example of cytotoxic ETP alkaloid which was initially isolated from the marine fungus *Emericella nidulans*. It is also found in other fungi such as *E. striata*, *E. foveolata*, *E. quadrilineata*, *etc.*[15] This compound have been tested against T47D, HeLa, and WiDr and the best activity was observed toward T47D cells (breast cancer) with IC_{50} of 0.25 μM. Flow cytometry analysis showed this compound induced apoptosis in T47D cells.[15] The mechanism of toxicity of compound **7** was found to be deformation of the mitochondrial structure by inhibiting the ATP synthesis by uncoupling of oxidative phosphorylation along with depression of respiration in isolated mitochondria.[54]

Pyridone Alkaloids

In fungi, polyketides (PKs) and nonribosomal peptides (NRPs) are involved in the biosynthesis of pyridone alkaloids which are present in the tetracyclic or tricyclic form.[55,56] Chaunolidone A (**8**), a pyridinone was isolated from marine fungus *Chaunopycnis* sp. (CMB-MF028) along with its analogs. The isolated



Penicilazaphilone B
1

Penicilazaphilone C
2

Figure 2. Cytotoxic penicilazaphilones ($IC_{50} < 1$ μM) from fungus (2014–2021).

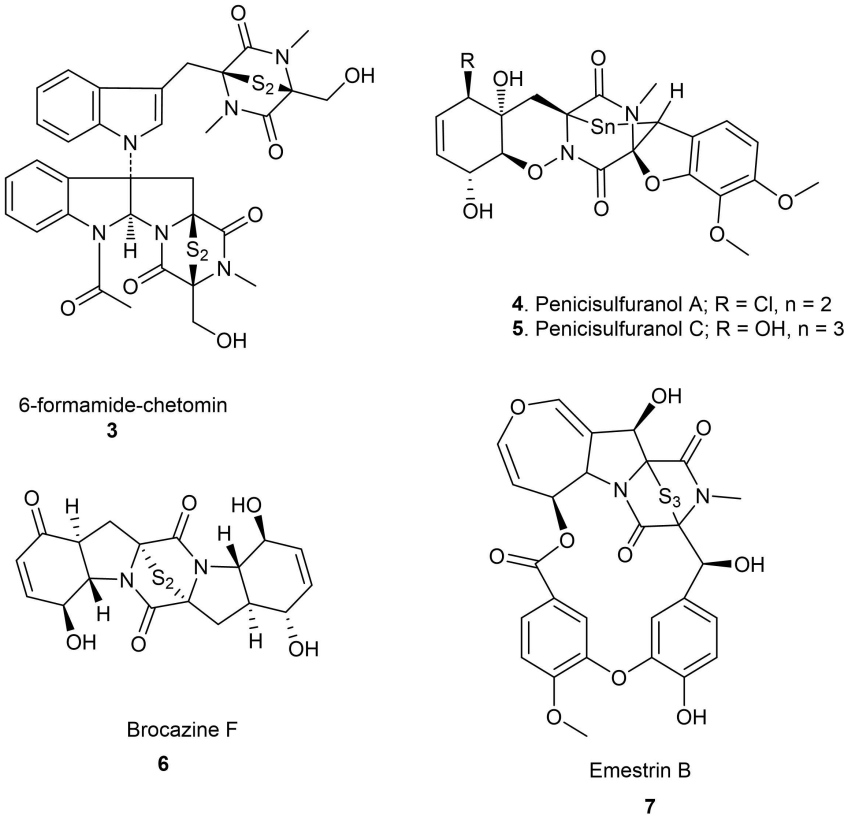


Figure 3. Cytotoxic disulfide bridged diketopiperazine alkaloids ($IC_{50} < 1$ μM) from fungus (2014–2021).

molecules were tested against human colorectal adenocarcinoma (SW620), non-small cell lung carcinoma (NCI-H460), and cervical carcinoma (KB3-1). This compound **8** has shown selective inhibitory activity against human non-small cell lung carcinoma cells with IC_{50} 0.09 μM. The percentage extractive yield of (**8**) was 0.38% w/w.[14] Further, N-hydroxy-apiosporamide (**9**) was isolated from lichen *Cladonia* sp. associated fungus *Apiospora montagnei* which was grown on rice media. The percentage extractive yield of compound **9** was found to be 0.56% w/w. Compound **9** has demonstrated an excellent inhibitory action at 0.2 μM against L5175Y mouse lymphoma cells.[22]

Orbiocrellin B (**10**) from pathogenic fungus *Orbiocrella* sp. *BCC 33248* found in *Coccoidea* insect Hemiptera have shown potent cytotoxicity ($IC_{50} = 0.77$ μM) against NCI-H187 cells. The obtained yield was 0.12% w/w when fermented under static conditions for 33 days at 25 °C with M102 medium. In total, six molecules were isolated from this fungus which belongs to N-hydroxypyridone alkaloids, chromone derivatives, and tetrahydroxanthones. The cytotoxicity of these molecules were tested on NCI-H187 cells, MCF-7 and KB cells (*Table 1*; *Figure 4*).[19]

Terpenoids

Ophiobolins

Ophiobolins are sesterterpenoids containing tricyclic 5-8-5 carbotricyclic skeleton and these compounds were first reported from *Bipolaris* sp.[57] Initially ophiobolins were known to be phytotoxins as it seems to produce disease syndrome in plants. From 1999 to 2016, many ophiobolins have been reported including their subtype/derivative which is further divided into various subgroups. Most of them were isolated from the genus *Aspergillus* and *Bipolaris*. It has been observed that more than 50% of ophiobolins showed anti-cancer activity. Among those, ophiobolin A is a potent anti-cancer molecule active against 26 human cancer cell lines. It was also found to be active in mouse GBM (glioblastoma multiforme) and melanoma models. The structure-activity relationship study of ophiobolin A revealed important structural features such as a) configuration at C-6 position, b) attachment

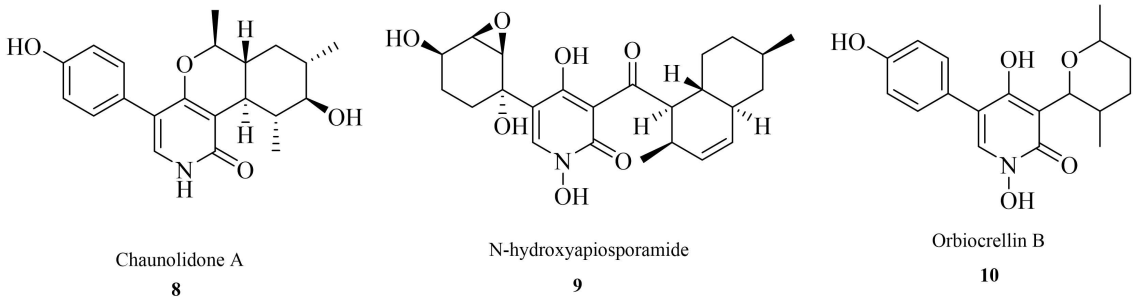


Figure 4. Cytotoxic pyridone alkaloids (IC_{50} < 1 μM) from fungus (2014–2021).

of hydroxy group at C-3, C-6 and C-14 position, and c) position of tetrahydrofuran ring in between C-14 to C-17 and C-5 to C-21 position. The findings suggested that the hydroxy group at C-6 position showed 8–9 times greater efficiency in inhibiting the growth of both K562 and HepG2 cells and the tetrahydrofuran ring between C-5 to C-21 suppressed the cytotoxic activity. The reported mechanism of action is via apoptosis, necrosis, and paraptosis but detailed investigations are needed.[58] In this review, we have covered the cytotoxic ophiobolins reported after 2014 including ophiobolins X, ophiobolins Y, 21-dehydro-ophiobolin U, ophiobolin Z, 21-*epi*-ophiobolin Z, 21-*epi*-ophiobolin O and 21-deoxyophiobolin K from mangrove fungi *Aspergillus ustus* 094102. These compounds were tested against G3K, MD-MBA-231, MCF-7, MCF-7/Adr, A549, HL-60 and 21-*epi*-ophiobolin O (**11**) found to be potent against A549, HL-60 with IC_{50} value 0.6 and 0.8 μM, respectively. The percentage extractive yield of compound **11** was 0.008 % w/w.[46]

Further, *Aspergillus flocculosus*, a marine fungus when cultured on rice media for three weeks lead to the isolation of five ophiobolins, 14,15-dehydro-6-*epi*-ophiobolin K, 14,15-dehydroophiobolin K, 14,15-dehydro-6-*epi*-ophiobolin G, 14,15-dehydroophiobolin G, 14,15-dehydro-(*Z*)-14-ophiobolin G. All the compounds were tested against HCT-15, NUGC-3, NCI-H23, ACHN, PC-3, MDA-MB-235 cancer cell lines and 14,15-dehydro-6-epi-ophiobolin K (**12**) and 14,15-dehydro-ophiobolin K (**13**) showed potent activity against tested cancer cell lines (*Table 1*). While, 14,15-dehydro-6-*epi*-ophiobolin G (**14**) was active against HCT-15, and NUGC-3 cell lines with IC_{50} 0.96 and 0.88 μM, respectively. The percentage extractive yield of **12**, **13** and **14** were found to be 0.03 % w/w, 0.006 % w/w, and 0.01 % w/w, respectively. (*Table 1*; *Figure 5*)[18]

Sesquiterpenes

Sesquiterpenes having three isoprene units are well-known for their anticancer properties.[59] Aspergillusone D (**15**) is a candinane type sesquiterpenoid (*Figure 6*) that has shown potent cytotoxic activity against A549. This compound was isolated from an endophytic fungus *Aspergillus clavatus*.[29] There are 20 sesquiterpenes reported from endophytic fungus *Pseudolagarobasidium acaciicola* from tree *Bruguiera gymnorrhiz*. All these compounds were screened for cytotoxic activity against HuCCA-1, A549, MOLT-3, HepG2, HL-60, MDA-MB231, T47D, HeLa, MRC-5 and among all, 7-epi-merulin B (**16**) showed potent response against HL-60 with IC_{50} 0.28 μM.[30] Similarly,

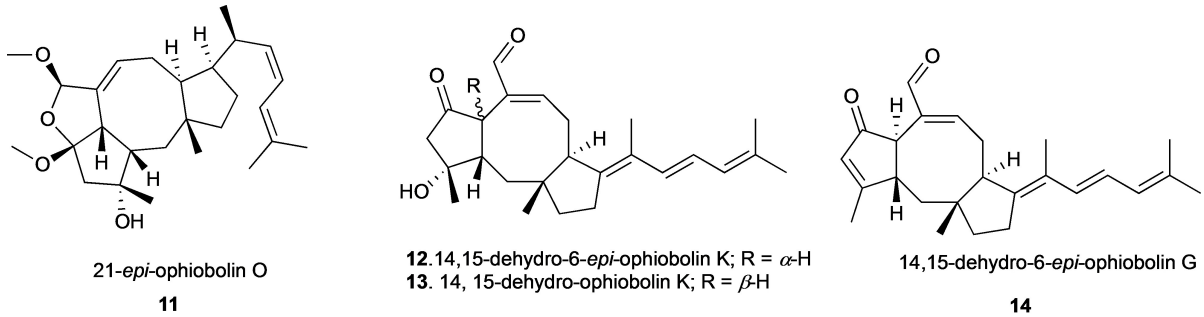


Figure 5. Cytotoxic ophiobolins (IC_{50} < 1 μM) from fungus (2014–2021).

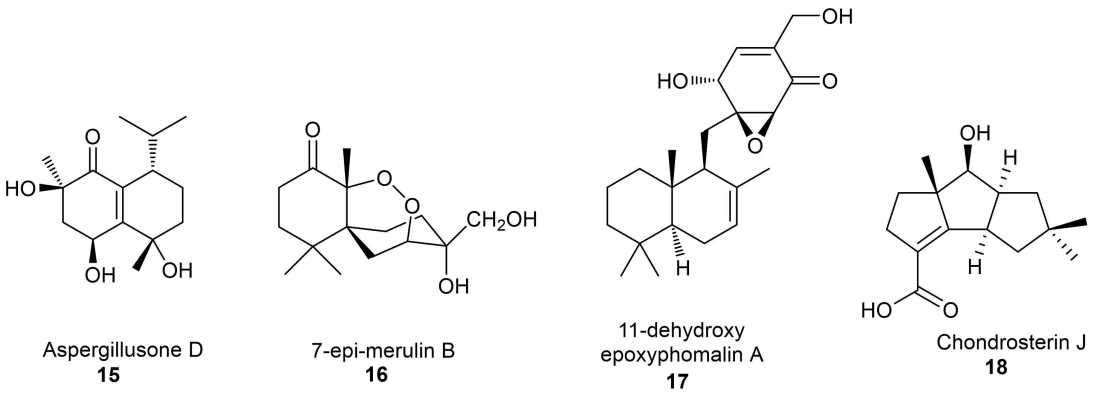


Figure 6. Cytotoxic sesquiterpenes ($IC_{50} < 1$ μM) from fungus (2014–2021).

epoxyphomalin analog named 11-dehydroxy epoxyphomalin A (**17**) from endophytic fungus *Peyronellaea coffeae-arabicae* FT238 of *Pritchardia lowreyana* plant showed IC_{50} value 0.5 μM against the OVCAR3 cell lines.[32] Chondrosterins I & J have been extracted from *Chondrostereum* sp. residing in the tissue of soft coral *Sarcophyton tortuosum*. Out of which, chondrosterins J (**18**) showed potent inhibitory activity against CNE-1 and CNE-2 cancerous cell lines.[16] The yield of **18** was found to be 0.05% w/w. An enantioselective total synthesis of **18** via intermolecular aldol reaction has been reported with 19.4% yield.[60]

Triterpenoids

Triterpenoid is a major class of Natural Products having over 20,000 known members. Squalene is a 30 carbon intermediate initiating with isopentenyl pyrophosphate leading to triterpenoids which include protostanes, lanostanes, holostanes, cycloartanes, dammaranes, euphanes, tirucallanes, tetranortriterpenoids, quassinoids, lupanes, oleananes, friedelanes, ursanes, hopanes, isomalabaricanes, and saponins.[61] Integracides F (**19**) and G (**20**) have shown potent cytotoxicity against SKOV-3 at IC_{50} 0.23 and, 0.17 μM, respectively (*Figure 7*). These tetracyclic triterpenoids were isolated from endophytic fungi *Fusarium* sp. grown on rice containing media. This fungus was found in the roots of *Mentha longifolia* L. collected from Saudi Arabia.[27] In 2016, integracides H–J were isolated from the same fungus *Fusarium* sp. among which, integracides H (**21**) showed potent growth inhibitory activity against BT-549, SKOV-3, KB cell lines at IC_{50} values 1.82, 1.32, 0.18 μM, respectively. In this study, doxorubicin (IC_{50} value 2.54 μM) was used as a positive control.[28]

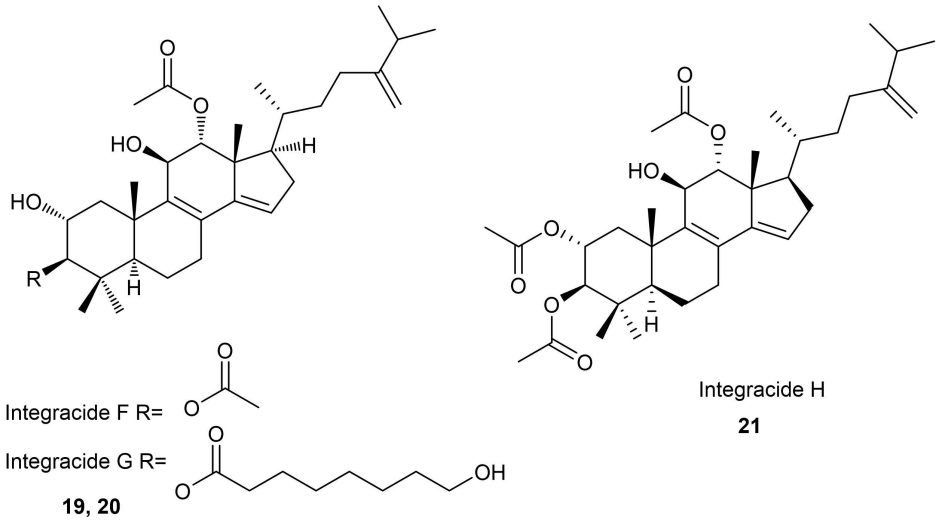


Figure 7. Cytotoxic triterpenoids ($IC_{50} < 1$ μM) from fungus (2014–2021).

Azanthraquinones

Cytotoxic anthraquinones, Torrubiellins A & B were initially found in the endophytic fungus *Torrubiella* sp. Bcc 28517.[62] Later, in 2015, Torrubiellin B (**22**) was extracted from *Acremonium* sp. from plant *Sonneratia caseolaris*. This fungus was cultured for 4 weeks using a solid rice medium leading to the 2% w/w extractive yield of **22** which was greater than the earlier *Torrubiella* sp. Bcc 28517 culture (0.46% w/w). Compound **22** was evaluated for anti-cancer activity against several cancerous cell lines like Cal27, Kyse510, HCC38, MDA-MB-231, A2780 and found potent against Cal27, HCC38, A2780 with IC_{50} 0.3, 0.4, 0.3 μM, respectively, in comparison to standard cisplatin, which showed IC_{50} 2.9, 3.5, 1.5 μM against the same cell lines.[41]

Further, 7-Desmethylscorpinone (**23**) and 7-desmethyl-6-methylbostrycoidin (**24**) from endophytic fungus *Fusarium solani* have shown potent cytotoxicity. The fungus was isolated from the roots of *Aponogeton undulatus Roxb* found in Natore, Bangladesh in 2013. The percentage extractive yield of **23** and **24** was 0.11% w/w and 0.16% w/w, respectively. The cytotoxic effect of these compounds was studied against HeLa cervical carcinoma, MIA paca2, MDA MB 231 breast cancer, NCI H1975, and WI38. Compound **23** was found active against HeLa cervical carcinoma, MIA paca2, and NCI H1975 with IC_{50} value 0.96, 0.98, 0.61 μM, respectively. Compound **24** was found to be active against HeLa cervical carcinoma, MIA paca2, MDA MB 231 breast cancer, NCI H1975 with IC_{50} value 0.71, 0.73, 0.64, 0.34 μM, respectively. An *in-silico* study showed the ability of **23** and **24** to construct a stable complex with mixed AT/GC DNA sequences with binding energies of −8.3 and −7.9 kcal/mol, respectively. However, further mechanistic studies are needed to reconfirm the abilities of **23** and **24** to intercalate DNA to produce toxicity against cancerous cell lines.[44] Variecolortides isolated from *Eurotium* sp., an endolichenic fungal strain present in lichen *Cladina grisea* have potential as an anti-cancer agent. Racemic mixture of variecolortide B (**25a** and **25b**) (*Figure 8*) have shown potent inhibitory caspase-3 activity (IC_{50} 0.8 ± 0.2 μM) but has a poor percentage extractive yield (0.06% w/w).[23]

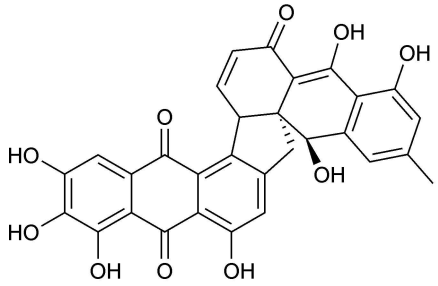
Macrolides

Macrolides are the largest subgroup of the polyketide class of Natural Products having acyl-CoA as a precursor.[63] Epiroridine acid (**26**), a trichothecene macrolide, has shown potent cytotoxicity against SF-268, MCF-7, NCI-H460, HepG-2 cell lines with IC_{50} values of 0.751, 0.170, 0.360, 0.38 μM, respectively. This compound was isolated from endophytic fungus *Myrothecium roridum* A553 (yield-0.27% w/w) obtained from plant *Pogostemon roridum* A553.[45] The mechanistic studies carried out on the HepG-2 cell lines showed that **26** induces apoptosis via activation of caspase-3 and caspase-9. Also, it leads to disruption of the mitochondrial membrane along with up-regulation of Bax gene expression, down-regulation of Bcl-2 gene expression.[64]

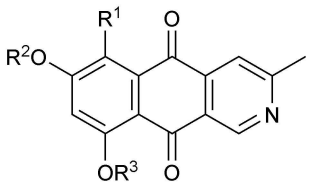
7-O-methylnigrosporolide (**27**), a 14-member macrolide, was isolated from endophytic fungi *Pestalotiopsis microspore* from fruit *Drepanocarpus lunatus* (Fabaceae) having yield 0.01% w/w. This macrolide was screened against the human ovarian cancer cell lines A2780 and mouse lymphoma cell lines L5178Y and showed potent activity against lymphoma cells with IC_{50} 0.7 μM.[35] A 13 step total synthesis method has been reported for the preparation of **27** with the starting material *D*-mannitol and 5-hexenol leading to 10.5% w/w yield (*Table 1*; *Figure 9*).[65]

Miscellaneous

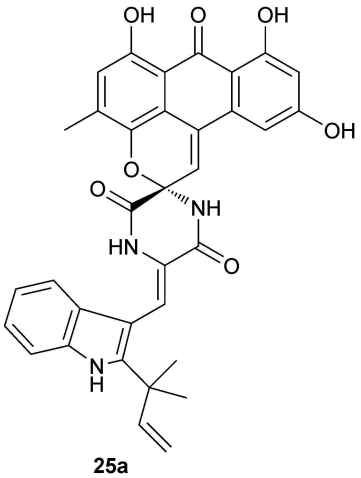
An investigation of endophytic fungus *Penicillium* sp. FJ-1 of *Avicennia marina* revealed two antitumor molecules namely 15-hydroxy-6α,12-epoxy-7β,10αH,11βH-spiroax-4-ene-12-one (HESEO) (**28**) and 4-(2′,3′-dihydroxy-3′-methylbutanoxy)phenethanol. Both were tested against the Human lingual carcinoma cell lines (Tca8113), Osteosarcoma cell lines (MG-63), and human liver cell lines (WRL-68). The potent antiproliferative activity of **28** was observed against the MG-63 cell lines with IC_{50} 0.055 μM.[25] The percentage extractive yield of **28** was 0.05% w/w. HESEO induces apoptosis of MG-63 through increasing expression of *NF-$_K$B p-P65 and PUMA*, and mitochondrial dysfunction.[66] PM181110 (**29**), a depsipeptide has shown an interesting cytotoxic profile and tested against 40 human tumor cell lines including cells of various organs like bladder, colon, head and neck, lung, mammary, melanoma, ovarian, pancreas, pleuramesothelioma, renal, sarcoma, and uterus. It was highlighted that pancreatic cancer cells (PAXF 546L) and lungs cancer cells (LXFA 526L) were highly sensitive towards compound **29** with IC_{50} 0.016 and 0.021 μM. It was further screened *ex vivo* against 24 human tumor xenografts models and found to be



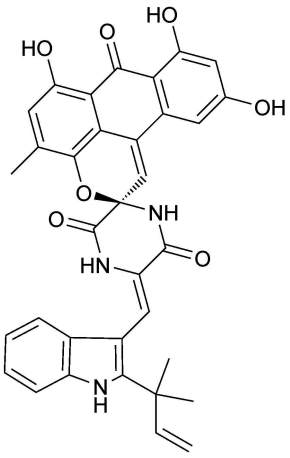
Torrubiellin B
22



23. 7-desmethylscorpinone; R^1 = H, R^2 = H, R^3 = CH_3
24. 7-desmethyl-6-methylbostrycoidine; R^1 = OCH_3, R^2 = H, R^3 = H



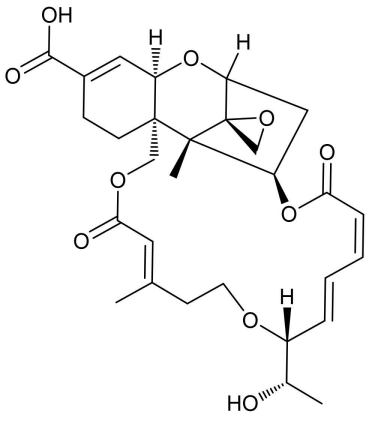
25a



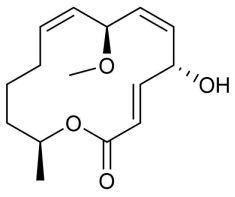
25b

Variecolortide B

Figure 8. Cytotoxic azanthroquinone (IC_{50} < 1 μM) from fungus (2014–2021).



Epiroridin acid
26



7-O-methylnigrosporolide
27

Figure 9. Cytotoxic macrolides (IC_{50} < 1 μM) from fungus (2014–2021).

potent against bladder cancer BXF 1218 with IC_{50} 0.03 μM. However, *in vivo* studies showed no antitumor activity which needs to be confirmed by changing dose regimens and formulations. Compound **29** was isolated from fungi PM0509732 obtained from the

tissue leaves of *Pongamia pinnata* (L) with 4% w/w extractive yield.[26]

Rhytidenone H (**30**), a spirobisnapthalenes type of compound obtained from the mangrove associated fungi *Rhytidhysteron rufulum* AS21B showed potent inhibitory activity against Ramos and H1975 cell lines at IC_{50} values 0.018 and 0.252 μM, respectively. The yield of compound **30** was found to be 0.03% w/w.[40] A benzamide derivative, fusarithioamide A (**31**) was isolated from fungi *Fusarium chlamydosporium* residing in the leaves of *Anvillea garcinii* (yield-0.12% w/w). This compound was tested on KB, BT-549, SK-MEL, and SKOV-3 cell lines and found to be potent against BT-549 and SKOV-3 with IC_{50} 0.4 and 0.8 μM, respectively. The positive control was doxorubicin.[42] Further investigation of this fungi resulted in potent fusarithioamide B (**32**) with a yield of 0.05% w/w.[42] Emericellipsin A (**33**) is a lipopeptaibol type compound isolated from soil fungi *Emericellopsis alkalina*. The compound exhibited a potent cytotoxic effect on HeLa cell lines with EC_{50} <0.5 μM.[20] A cyclic pentapeptide, disulphide cyclo-(Leu-Val-Ile-Cys-Cys) named Malformin E (**34**) has shown remarkable cytotoxicity against the MCF-7 cell lines with IC_{50} value 0.65 μM which is reported from the endophytic fungus *Aspergillus tamari* from roots of *Ficus carica*. The yield of **34** was found to be 0.02% w/w.[33]

α-Pyrone derivatives named phomones have shown anti-cancer properties. Phomone C–F were isolated with 4% w/w yield from endophytic fungus *Phoma* sp. *YN02-P-3* from plant *Sumbaviopsis albicans* J. Out of these compounds, phomone D (**35**) was found to be potent against the HL-60 cell lines with IC_{50} 0.65 μM. The structural activity relationship of **35** revealed that the acetyl group at the 10th and 12th positions play a dominant role in inhibitory activity against cancerous cells (*Table 1*; *Figure 10*).[34] Macrophin (**36**) was discovered from a fungus *Phoma macrostoma* residing inside the tissue of the plant *Glycyrrhiza glabra* Linn. The yield of 36 was 8.08% w/w. This compound (**36**) was found to be potent pancreatic cancer with IC_{50} 0.9 μM. Based on several mechanistic studies of **36**, it was hypothesized that programmed dysregulated cell death occurs in cancerous cells is due to apoptosis by S phase arrest.[37] A study on *Alternaria phragmospora*, an endophytic fungi resulted into isolation of four molecules named as 5-butyl-4-methoxy-6-methyl-2*H*-pyran-2-one, 5-(1-hydroxybutyl)-4-methoxy-6-methyl-2*H*-pyran-2-one,4-methoxy-6-methyl-5-(3-oxobutyl)-2*H*-pyran-2-one, and 4-hydroxy-6-methyl-5-(3-oxobutyl)-2H-pyran-2-one. All compounds were screened for their growth inhibitory activity against HL-60 and K562 and among all, 4-methoxy-6-methyl-5-(3-oxobutyl)-2H-pyran-2-one (**37**) showed anti-cancer activity at IC_{50} 0.9 μM against HL-60. The yield of compound **37** was 0.03% w/w.[36] A five steps synthetic method for preparation of 37 has been reported from 4-hydroxy-5-methoxycarbonyl-6-methyl-2-pyrone.[67]

An additional potent cytotoxic molecule, 3-*epi*-waol A (**38**), a γ-lactone extracted from fungus *Libertella blepharis* found in the inner tissue of leaf of *Olyra latifolio*. The yield of compound **38** was 0.03% w/w. Compound **38** was evaluated against 12 cell lines includes showing inhibitory activity against H460 with an IC_{50} value of 1.0 μM.[38] Phialomustins A–D, azaphilone derivatives, have been isolated from endophytic fungi *Phialophora mustea* from plant *Crocus sativus*. Antiproliferative activity of phialomustins was checked against pancreatic, lung, colon, and human breast cancer cell lines using MTT assay and found phialomustins B (**39**) as a potent molecule with IC_{50} 1.0 μM. The yield of compound **39** was 8.33% w/w.[39] Myceliothermophins polyketide-amino acid hybrid natural compounds were isolated from *Thermothelomyces thermophilus* ATCC 42464. Myceliothermophins A (**40**), E (**41**), and F (**42**) showed potent toxicity against DLD-1, Hep3B, and HGC-27 cell lines.[21]

Discussion

A review on cytotoxic fungal metabolites from 1964 to 2013 has very finely explained the role of fungi in anticancer discovery highlighting potential cytotoxic molecules like cytochalasins, fusicoccin A, ophiobolins, ophiobolin O, ophiobolin A, phyllostictin A, tryprostatins, tryprostatin A, halenaquinones, TAN1496 A–E, gliotoxins, macrosphelide, cordycepin, panepoxydone, cycloepoxydone, oxaspirodion, caffeic acid phenethyl ester.[11] This review has described molecules having *in-vitro* IC_{50} value below 1 μM and isolated from the fungal origin after 2014. This report describes 42 potent molecules, out of which 9 are from marine fungus, 2 from endolichenic fungi, 5 from terrestrial fungi and 26 from endophytic fungi. All the available data of these molecules are described including their source, scaffold chemistry, percentage extractive yield, fermentation conditions, mechanism of action and structure-activity details.

The comprehensive analysis of these compounds showed that maximum data required for the drug discovery process is available for penicisulfuranol A (**4**), a disulfide bridged alkaloid, which has shown potent

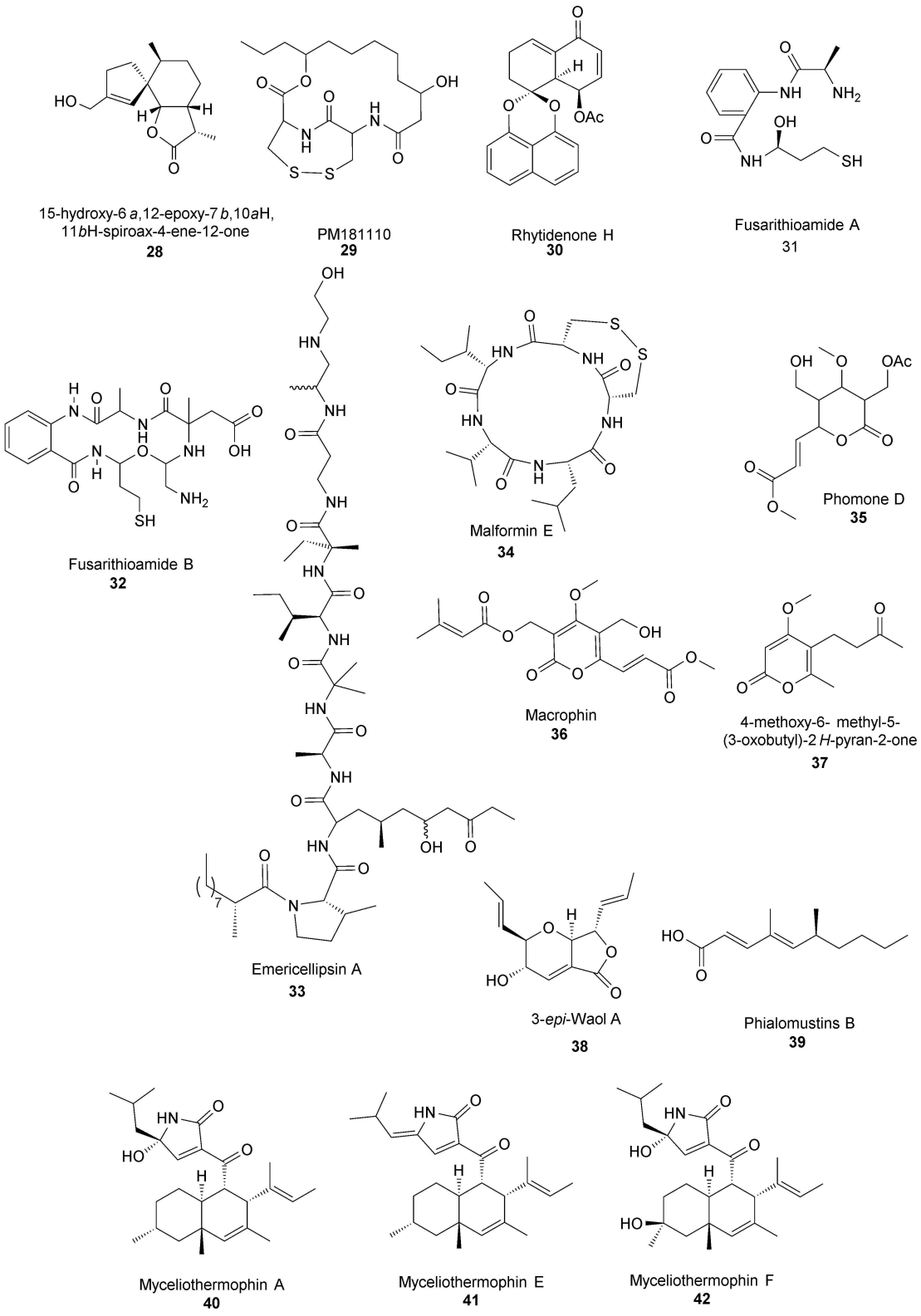


Figure 10. Cytotoxic compounds belong to miscellaneous group ($IC_{50} < 1$ μM) from fungus (2014–2021).

in-vitro cytotoxicity against HeLa (IC_{50} = 0.5 μM) and HL60 (IC_{50} = 0.1 μM) cell lines. It acts through inhibiting the C-terminal of HSP-90 heat shock protein. A synthetic method using molybdenum mediated oxidation has been developed for the production of this molecule (**4**). So, this molecule should be given priority and further evaluated via *in vivo* study and toxicity studies to proceed further in the drug discovery pipeline. Penicilazaphilone C (**2**) is another candidate from this structural class which has shown cytotoxic activity against human gastric cancer cells SGC7901 with IC_{50} value 0.72 μM and significant tumor reduction in tumor induced BALB/c-nude mice, *in vivo* model. The molecule (**2**) acts via blocking the notch signalling pathway which is one of the key pathway in deciding the cell fate. However, further studies like pharmacokinetic and toxicity profiling along with formulation development are still pending.

Ophiobolins are also an interesting class of secondary metabolites mostly produced by *Aspergillus* and *Bipolaris* species. A comprehensive report of Ophiobolin A and its derivatives has been given earlier.[68] 21-*epi*-ophiobolin O (**11**) has shown promising anticancer activity against adenocarcinoma and promyelocytic leukemia and detailed evaluations need to be carried out to exploit its full potential. In addition, 6 formamide chetomin, PM181110, N-hydroxyapiosporamide, 15-hydroxy-6α,12-epoxy-7β,10αH,11βH-spiroax-4-ene-12-one have shown potent *in-vitro* activity and should be thoroughly assessed by studies like *in vivo* evaluation, SAR, pharmacokinetic, toxicity, formulation aspects and clinical studies to convert them into leading anticancer drugs.

It is evident that in the last 8 years number of cytotoxic molecules have been reported from the fungal origin and the potent ones are elaborated in this report. Most of the potent compounds are isolated from endophytic fungi indicating their significant role in plant species with the hypothesis that either they produce these cytotoxic molecules to compete with other microorganisms or to support plants for their survival. Though the role of these compounds is not clear, it gives insights to researchers for the discovery of new drugs and in a few cases to find new targets for particular diseases specifically cancer.

Acknowledgements

We acknowledge the Department of Science and Technology for funding Project – DST/INT/SL/P-22/2016, Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, Government of India and National Institute of Pharmaceutical Education and Research (NIPER), Ahmedabad for providing the continuous support and facilities required for the work. We would also like to acknowledge Miss Sai Sowmya for support in conducting a literature survey of terrestrial fungus.

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution Statement

Chaitrali Shevkar has carried out the literature search on endolichenic fungi, performed data analysis and wrote the manuscript. Pranali Pradhan has collected the literature data on marine fungi, performed data visualization and wrote the manuscript. Ashwini Armarkar and Komal Pandey have collected the literature data related to endophytic fungi. Prof. Kiran Kalia and Prof. Priyani Paranagama were involved in idea generation and data analysis. Abhijeet S. Kate was instrumental in conceptualization, data analysis and further involved in revision and proofreading of the manuscript.

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Received December 7, 2021
Accepted March 3, 2022

