บทความวิชาการ

สารออกฤทธิ์ต้านเซลล์มะเร็งจากจุลชีพที่อาศัยอยู่กับพืช

Anticancer Agents from Plant-Associated Microorganisms

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Introduction

Natural products are metabolites and/or by-product naturally derived from plants, animals, or microorganisms. These products serve as principal compounds whose activities can be enhanced by manipulation through combinatorial and synthetic chemistry. From 1989 to 1995, over 60% of approved drugs and pre-new drug application candidates for cancer and infectious diseases were of natural origin [1].

Microorganisms are rich source of biologically active metabolites that find wide-ranging exploitation in medicine, agriculture, and industry. Originally, interest was placed on harmful microorganisms which cause disease or spoilage of food and beverages. However, it is now recognized that many microbes have essential roles in our ecosystem. Some of their metabolites are interesting candidates in pharmaceutical industry, for example, erythromycin [2], lovastatin (cholesterol lowering agent) [3], and cyclosporine (immunodepressant) [4].

At present, there has been increasing interest in chemical investigation of microorganisms in search for drug precursor due to the development of fermentation and screening technologies. The purpose of this review is to summarize the endophytic microorganism products as anticancer agents.

Endophytic Microorganisms

The term endophytes refer to bacterial or fungi that colonize in the interior of plant organs, but do not have any pathogenic effects on its host(s). In their symbiotic association, the host plant (macrophyte) protects and feeds the endophyte, while the endophyte in turn produces bioactive metabolites to enhance growth and competitiveness of the host and to protect it from herbivores and plant pathogens [5]. It appears that all higher plants are hosts to one or more endophytic microbes. These endophytic relationships may have begun to evolve from the time that higher plants first appeared on the earth, hundreds of millions of years ago. Evidence of plant associated microbes has been discovered in the fossilized tissues of stems and leaves [6]. As a result of these long-held associations, it is possible to imagine that some of these endophytic microbes may have devised genetic systems allowing for the transfer of information

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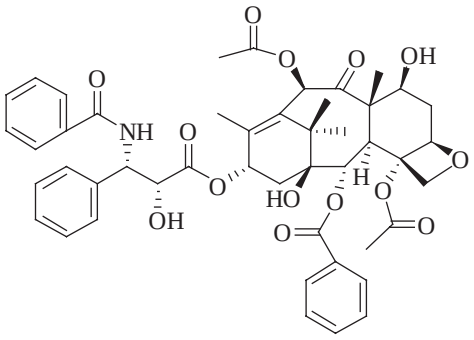
between themselves and the higher plant [7]. Obviously, this would permit a more rapid and reliable mechanism for the endophyte to deal with everchanging environmental conditions or environmental stress and perhaps allow for more compatibility with the host plant. Some of these endophytes may be producing bioactive substances that may be involved in a host-endophyte relationship. During the past two decades, over 100 endophytic microorganisms have been cultured and subjected to detailed investigations leading to the chemical characterization and biological evaluation of a large number of natural products, many of which have been shown to have novel structures and interesting biological activities [8]. Current interest in natural products from endophytic microorganisms is evident from the unique chemical structures acquired through evolution. Thus, there exists the potential of harvesting novel bioactive natural compounds from such plant-associated microorganisms. This is evident from characterization of over 400 natural products to date, of which most have novel structures and useful biological activities, from 128 plant-associated microorganisms [8].

Anticancer Agents from Plant-Associated Agents

Paclitaxel (Taxol), a highly functionalized diterpenoid, originally isolated from the Pacific yew tree *Taxus brevifolia*. Its unique mode of action, of preventing the depolymerization of tubulin during the processes of cell division, made it a huge success clinically and commercially. Taxol (**1**) and some of its derivatives represent the first major group of anticancer agents that are produced by endophytes. This compound is the world's first billion-dollar anticancer drug. The original target diseases for this compound were ovarian and breast cancers, but now it is used to treat a number of other human tissue-proliferating diseases as well [1].

To overcome its unacceptably low yield, taxol was produced in *in vitro* culture by a new endophytic fungus, *Taxomyces andreanae*, which was isolated from a Pacific yew *T. brevifolia* in Montana, USA. Since then, a variety of endophytic fungi isolated from yew trees and other plants such as *T. brevifolia, T. wallachiana, T. yunnanensis, T. baccata, T. mairei, Taxodium distichum, Torreya grandifolia,* and *Wollemia nobilis* have been reported to be capable of producing taxol and/or taxane derivatives [1].

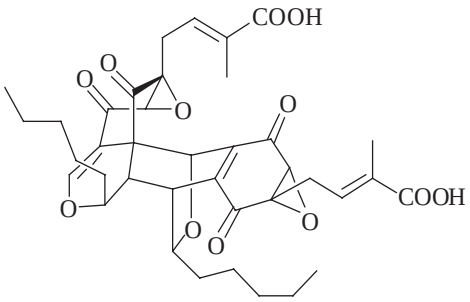
The greatest prospect of making microbial taxol a commercial reality may be the discovery of endophytes that make large quantities of one or more taxanes that could then be used as platforms for the organic synthesis of taxol or its anticancer relatives [1].



Taxol (**1**)

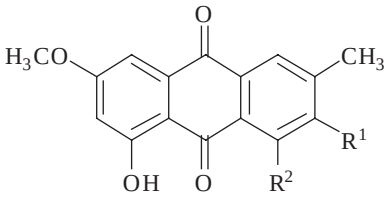
Torreyanic acid (**2**), a selectively cytotoxic quinone dimer, was isolated from the endophytic fungus *Pestalotiopsis microspora.* It (**2**) was tested in several cancer cell lines, and it demonstrated 5 to

10 times more potency in those cell lines that are sensitive to protein kinase C (PKC) agonists and causes cell death by apoptosis [9]. Recently, a complete synthesis of torreyanic acid (**2**) has been successfully completed using the application of a biomimetic oxidation-dimerization cascade [10].

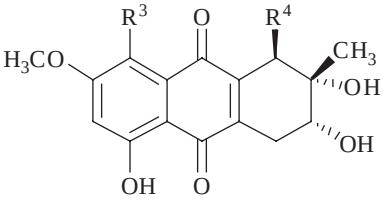


Torreyanic acid (**2**)

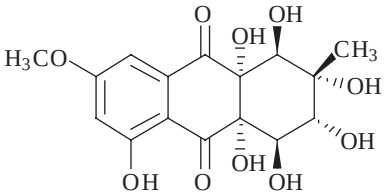
Six known metabolites, 7-methoxy-2-methyl-3,4,5-trihydroxyanthraquinone (**3**), physcion (**4**), macrosporin (**5**), deoxybostrycin (**6**), altersolanol B (**7**) and dactylariol (**8**), together with a new hexahydroanthraquinone named pleospdione (**9**) were isolated from the culture of *Pleospora* sp. IFB-E006, an endophytic fungus residing in the normal stem of *Imperata cylindrical* (Gramineae). Compounds **6-8** exhibited relatively high cytotoxic activities with IC_{50} values of 0.8 and 1.3 μg/mL, respectively, for compound 8, against human colon cancer (SW1116) and leukemia (K562) cell lines. However the cytotoxic activities of compounds **3, 4** and **9** were poor or moderate. These compare well to the IC_{50} value of 6.0 μg/mL for 5-fluorouracil used as a positive control [11].



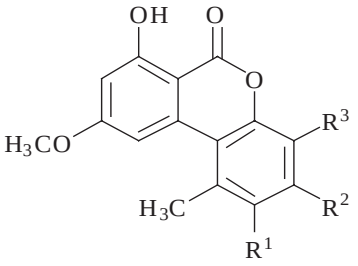
(**3**) $R^1 = R^2 = OH$
(**4**) $R^1 = H$, $R^2 = OH$
(**5**) $R^1 = OH$, $R^2 = H$



(**6**) $R^3 = OH$, $R^4 = H$
(**7**) $R^3 = R^4 = H$
(**8**) $R^3 = H$, $R^4 = OH$



(**9**)



(**10**) R^1 = Cl, R^2 = OMe, R^4 = H
(**11**) R^1 = H, R^2 = OMe, R^3 = OMe
(**12**) R^1 = H, R^2 = OMe, R^3 = OH
(**13**) R^1 = H, R^2 = OH, R^3 = H

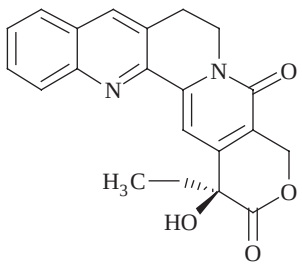
Table 1 *In vitro* cytotoxicity of **3, 4,** and **6 - 9** against SW1116 and K562 cells. The IC_{50} values (μg/mL) are presented [11].

	3	**4**	**6**	**7**	**8**	**9**	**5-fluorouracil**[a]
SW1116	> 100	> 100	5.8 ± 0.5	3.3 ± 0.3	0.8 ± 0.1	46.2 ± 6.0	6.0 ± 1.5
K562	> 100	> 100	3.1 ± 0.3	3.7 ± 0.5	1.3 ± 0.3	58.8 ± 4.7	6.5 ± 0.7

[a]Used as a positive control.

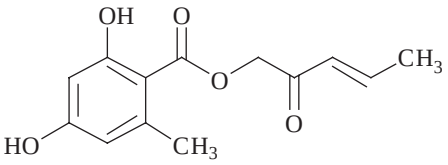
Two novel 6*H*-dibenzo[*b,d*]pyran-6-one derivatives, graphislactone G (**10**) and graphislactone H (**11**), together with graphislactone A (**12**) and alternariol monomethyl ether (**13**) were isolated from *Cephalosporium acremonium* IFB-E007, an endophytic fungus from roots of *Trachelospermum jasminoides* (LINDL.) LEM. Anticancer tests showed that compounds **10-13** had pronounced activities against SW1116 cell with IC_{50} values of 21, 12, 8.5, and 14 μg/mL, respectively [12].

Camptothecin (**14**), a pentacyclic quinoline alkaloid, was isolated from endophytic fungus (RJMEF001) in the inner bark of the plant *Nothapodytes foetida* from the Western coast of India. The fungus, which belongs to the family Phycomycetes, produced the anticancer drug lead compound; camptothecin (**14**). It was also compared with an authentic example for its biological activity against a number of human cancer cell lines (A-549 for lung cancer, HEP-2 for liver cancer, OVCAR-5 for ovarian cancer) [13]. Camptothecin and its derivatives showed strong antineoplastic activity. The drug is already used in China for the treatment of skin diseases. Hycamtin (topotecan) and Camptosar (irinotecan), semisynthetic derivatives of **14**, have been employed clinically for the treatment of ovarian and colon cancers. Compound **14** is also used as an insect chemosterilant, a plant regulator, and an inhibitor of the herpes virus. In addition, compound **14** prevents the replication of the influenza virus [13].



Camptothecin (**14**)

Globosumone A (**15**) and globosumone B (**16**), newly orsellinic acid esters from *Chaetomium globosum* endophytic in mormon tea *(Ephedra fasciculata)* exhibited moderate cytotoxicity against various cell lines, for example, non-small cell lung cancer (NCI-H460), breast cancer (MCF-7), CNS glioma (SF-268), pancreatic carcinoma (MIA Pa Ca-2), and normal human fibroblast cells (WI-38) [14].



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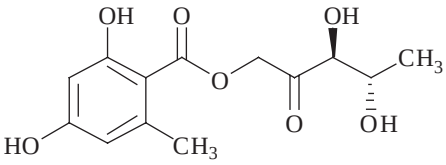
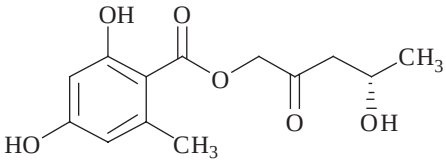


(**17**)



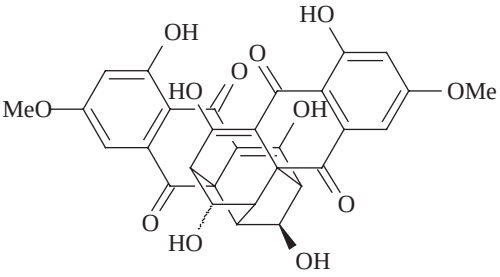
(**16**)

Table 2 Cytotoxicities of compounds **15-16** against a panel of four tumor cell lines and normal human primary fibroblast cells [14][a]

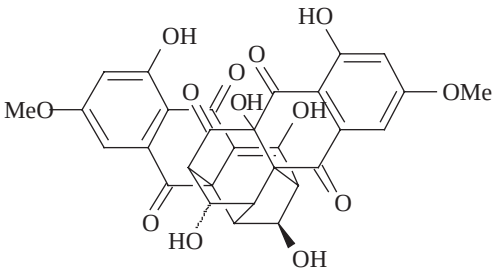
Compound	Cell line				
	NCI-H460	**MCF-7**	**SF-268**	**MIA Pa Ca-2**	**WI-38**
15	6.50	21.30	8.80	10.60	13.00
16	24.80	21.90	29.10	30.20	14.20
doxorubicin	0.01	0.07	0.04	0.05	0.30

[a]Results are expressed as IC_{50} values in μM; compound **17** was found to be inactive in all cell lines at 10.0 μg/mL.

The biochemical induction assay (BIA) is a rapid (colorimetric) bacterial assay used to identify compounds that damage DNA or inhibit DNA synthesis and thereby identify potential natural product anticancer agents. The use of BIA, which measures the induction of the SOS response in bacteria, led to the isolation of the bisanthraquinones cytoskyrins A (**18**) and B (**19**), from *Cytospora* sp. CR200, a fungal strain endophytic in *Conocarpus erecta*. The *cis, cis* conformation for cytoskyrin A (18) was confirmed by the X-ray crystallography structure and therefore, by analogy, assumed to be true for cytoskyrin B (**19**). It was likely that the cytoskyrins arise from the dimerization of 1, 3, 6, 8-tetrahydroxyanthraquinone via oxidation and condensation. Cytoskyrin A (**18**) showed strong BIA activity down to 12.5 ng in the standard BIA assay while cytoskyrin B (**19**) showed no significant BIA response at any of the concentrations tested (<50 μg) [**15**].

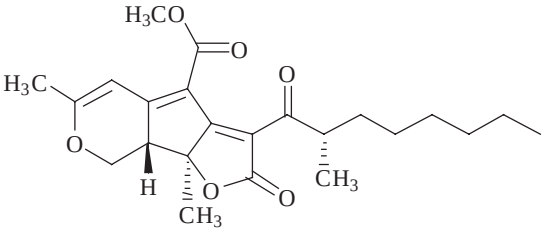


Cytoskyrins A (**18**)

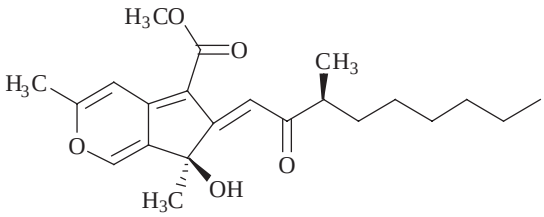


Cytoskyrins B (**19**)

Chemical investigation of *Aspergillus parasiticus*, an endophytic fungus in the inner bark of a coastal redwood tree, *Sequoia sempervirens*, also led to the isolation of sequoiatones A (**20**) and B (**21**). The absolute stereochemistry proof of sequoiatone A (**20**) was provided by X-ray crystallography [**16**]. Sequoiatones were tested against a panel of 60 different human tumor cell lines. They showed moderate and somewhat selective inhibition of human tumor cells, with greatest efficacy against breast cancer cell lines. Most of the GI_{50} (concentrations required to inhibit growth by 50%) were between 4 and 10 μM with LC_{50} > 100 μM.

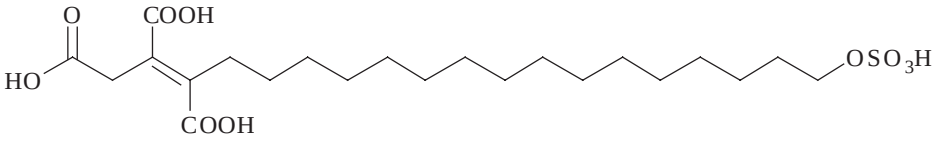


Sequoiatone A (**20**)



Sequoiatone B (**21**)

Oreganic acid (**22**), a tricarboxylated alkylsulfate and a specific inhibitor of farnesyl-protein transferase (FPTase) with an IC_{50} of 14 nM, was also found from the extract of an endophytic fungus isolated from leaves of *Berberis oregano* [17].



Oreganic acid (**22**)

Phomoxanthones A (**23**) and B (**24**), novel xanthone dimers, were isolated from the endophytic fungus *Phomopsi*s sp. BCC 1323. They exhibited significant activity against *Plasmodium falciparum* (K1, multi drug resistant strain) and against *Mycobacterium tuberculosis* (H37Ra strain), although weaker than standard drugs (Table 3). However, these compounds are also cytotoxic to two cancer cell lines (KB, BC-1) and to Vero cells [18].

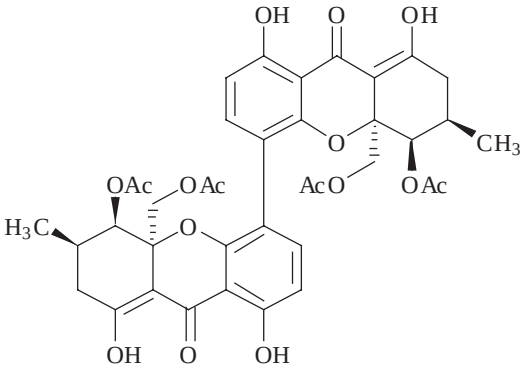
Table 3 Antimalarial and antitubercular activity and cytotoxicity of phomoxanthones A (**23**) and B (**24**) [18].

Compound	Antimalarial activity (IC_{50}, μg/mL)[a]	antitubercular activity (MIC, μg/mL)[b]	Cytotoxicity (IC_{50}, μg/mL)[c]		
	P. falciparum K1	*M. tuberculosis* H37Ra	KB cells	BC-1 cells	Vero cells
Phomoxanthone A	0.11	0.50	0.99	0.51	1.40
Phomoxanthone B	0.33	6.25	4.10	0.70	1.80

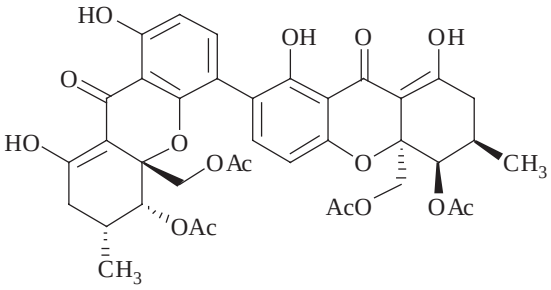
[a]The IC_{50} values of the standard antimalarial compounds, chloroquine diphosphate and artemisinin, are 0.16 and 0.0011 μg/mL, respectively.

[b]The MIC values of the antituberculosis drugs, isoniazide and kanamycin sulfate, are 0.050 and 2.5 μg/mL, respectively.

[c]The IC_{50} values of the standard compound, ellipticine, are 0.46 μg/mL for KB cells and 0.60 μg/mL for BC-1 cells.

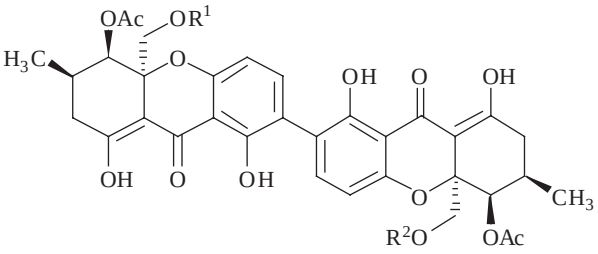


Phomoxanthone A (**23**)



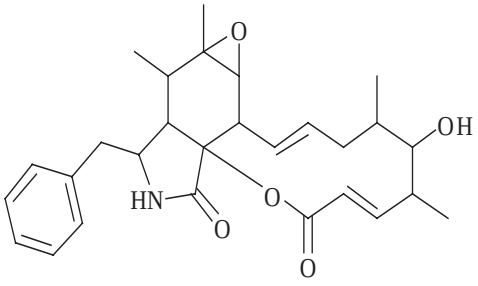
Phomoxanthone B (**24**)

Dicerandrols A (**25**), B (**26**), and C (**27**), new antibiotic and cytotoxic dimers, were isolated from *Phomopsis longicolla*, an endophytic fungus of the endangered mint *Dicerandra frutescens* [19]. These compounds (**25-27**) exhibited antibacterial activity against both *Staphylococcus aureus* and *Bacillus subtilis* but are inactive against the fungus *Geotrichum candidum* and the yeast *Saccharomyces cerevisiae* at 300 μg/disk (Table 4). They also possess modest activity in two human cancer cell lines, A549 (human lung tumor cells) and HCT-116 (human colon tumor cells).



(**25**) $R^1 = R^2 = H$
(**26**) $R^1 = Ac$, $R^2 = H$
(**27**) $R^1 = R^2 = Ac$

A series of new cytotoxic cytochalasins (**28**-**30**) were isolated from a culture of the endophytic fungus *Rhinocladiella* sp. They exhibited a broad spectrum of antibiotic and antitumor activity, phytotoxic activity, and inhibitory activity of HIV-1 protease. Although they are widely used as biological probes, their therapeutic application has been limited by their toxicity [20].



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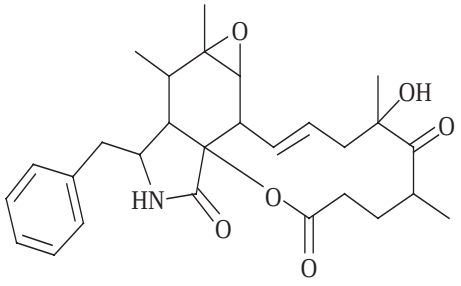

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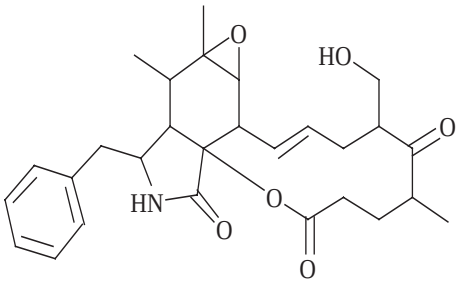

(**30**)

Conclusions and Future Prospects

This review highlight the fact that endophytes represent a rich and reliable source of bioactive and chemically novel compounds as anticancer agents. Metabolites derived from endophytes are offering us a great opportunity to evaluate not only new chemical classes of anticancer agents, but also novel and potentially activities. Thus, it should be noted that plant-associated microorganism is still not throughly explored, and there remains significant potential for novel discovery.

Table 4: Antimicrobial activity and cytotoxicity data for dicerandrols A, B, and C [19].

Compound	Zones of inhibition[a] (mm)		IC_{100} (μg/mL)	
	B. subtilis	*S. aureus*	A549	HCT116
Dicerandrol A	11.0	10.8	7.0	7.0
Dicerandrol B	9.5	8.5	1.8	1.8
Dicerandrol C	8.0	7.0	1.8	7.0
nystatin[b]	12.0			
neomycin[c]		9.0		
etoposide			30.0	125.0

[a]Zones of inhibition resulting from 300 μg/disk.

[b]Nystatin control disk contains 100 units (approximately 30 μg).

[c]Neomycin control disk contains 30 μg.

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