Chapter 5
Investigations of Lichen Secondary Metabolites with Potential Anticancer Activity

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Abstract Cancers figure among the leading causes of morbidity and mortality worldwide. In the past half a century, natural products have served us well in combating cancer. The main sources of these compounds are microorganisms, plants and marine organisms. Lichens as chemically significant biota represent a large group of symbiotic organisms of fungi (mycobiont) and algae (photobiont) comprising about 17,000 species, and are a source of diverse secondary metabolites.

This chapter focuses primarily on the anticancer properties of lichen secondary metabolites. We have reviewed various publications related to anticancer activity emphasizing results about specific lichen compounds. We have shown that various isolated lichen compounds often demonstrate significant inhibitory activity against various cancer cell lines at very low concentrations. Although lichens are a source for excellent anticancer active compounds, only a small number have been tested for their biological significance. This is our effort just another attempt to expand and deepen research in this area, especially on compounds that have shown promising results.

5.1 Introduction

Throughout history, natural products have afforded a rich source of compounds that have found many applications in the fields of medicine, pharmacy, and biology. Within the sphere of cancer, a number of important new commercialized drugs have been obtained from natural sources, by structural modification of natural compounds or by the synthesis of new compounds, designed following a natural compound as model. The search for improved cytotoxic agents continues to be an important line in the discovery of modern anticancer drugs. The huge structural diversity of natural compounds and their bioactivity potential have meant that several products isolated from plants, marine flora, and microorganisms can serve

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as “lead” compounds for improvement of their therapeutic potential by molecular modification. Over 70 % of anticancer compounds are either natural products or natural product-derived substances. On the other hand conjugation of toxic natural products to monoclonal antibodies or polymeric carriers can lead to more efficacious targeted therapies. Since less than 15 % of higher plants have been systematically investigated, the natural product research towards chemotherapy requires further attention and multi-scientific collaboration (Karikas 2010). Also, some herbal compounds have been subjected to clinical trials. This chapter focuses only on those herbal compounds originating from lichens whose anticancer effect was investigated. The aim of this chapter is to highlight the importance of lichen secondary metabolites with potential anticancer activity.

5.2 Anticancer Secondary Metabolites of Lichens

5.2.1 Lichens: Significance of Lichens and Lichen Secondary Metabolites

Generally, lichen metabolites can be divided into two groups: primary and secondary. Primary metabolites are proteins, lipids, carbohydrates, and other organic compounds involved in lichen’s metabolism and structure. Secondary metabolites, also known as lichen substances, are produced mainly by the fungus and secreted onto the surface of the lichen’s hyphae either in amorphous forms or as crystals. They often accumulate in the upper cortex or in specialized structures such as fruiting bodies (Fahselt 1994; Elix 1996). Lichens synthesize a great variety of secondary metabolites, many of which are unique. Approximately 1,050 secondary compounds have been identified to date (Stocker-Wörgötter 2008). They are produced by the mycobiont (Elix 1996; Huneck 1999) and accumulate in the cortex (such as atranorin, parietin, usnic acid, fungal melanins) or in the medullary layer (such as physodic acid, physodalic acid, protocetraric acid) as extracellular tiny crystals on the outer surfaces of the hyphae. The photobiont might also have an influence on the secondary metabolism of the mycobiont (Brunauer et al. 2006, 2007; Yamamoto et al. 1993; Yoshimura et al. 1994; Molnár and Farkas 2010).

Lichens had to evolve diverse biosynthetic pathways to produce such complex arrays of secondary metabolites: polyketide, shikimic acid, and mevalonic acid pathways. Most of the lichen substances are phenolic compounds. Polyketide-derived aromatic compounds, depsides, depsidones, dibenzofurans, xanthones, and naphthoquinones, are of great interest. Compounds from other pathways are esters, terpenes, steroids, terphenylquinones, and pulvinic acid (Fahselt 1994; Cohen and Towers 1995; Müller 2001; Brunauer et al. 2006, 2007; Stocker-Wörgötter and Elix 2002; Johnson et al. 2011; Manojlovic et al. 2012). So, many lichens and lichen products have proved to be a source of important secondary metabolites for food and pharmaceutical industries (Huneck 1999; Oksanen 2006)

and still hold a considerable interest as alternative treatments in various parts of the world (Richardson 1991). Also, we know more about these substances through experimental studies, but the functions of these compounds in the lichen symbioses are still poorly understood (Hager et al. 2008). They may impact biotic and abiotic interactions of lichens with their environment. In addition, they may help to protect the thalli against herbivores, pathogens, competitors, and external abiotic factors, such as high UV irradiation. A wide spectrum of biological potential is shown by the lichens, but they have been long neglected because of their slow-growing nature and difficulties in their artificial cultivation and have scarcely been studied from a biochemical perspective (Crittenden and Porter 1991; Yamamoto et al. 1995; Yamamoto 2000; Behera et al. 2003, 2004). Industrial-scale harvests of lichens are neither ecologically sensible nor sustainable and for many species are not feasible. The new technologies in molecular biology come in light for the direct access of lichen genomes to reveal and eventually to harvest the production of novel secondary metabolites (Miao et al. 2001).

Furthermore, lichen substances exhibit a great diversity of biological effects, including antimicrobial, antiinflammatory, analgesic, antipyretic, and antiproliferative and cytotoxic activities (Boustie and Grube 2005). However, relatively few lichen substances have been screened in detail for biological activity and therapeutic potential, principally due to difficulties in obtaining them in quantities and purities sufficient for structural elucidation and pharmacological testing (Muggia et al. 2009).

5.2.2 *Lichen Secondary Metabolites as Potential Anticancer Drugs (Some Earlier Studies)*

As noted above, over 1,050 secondary metabolites have been reported for lichens and cultured aposymbiotically mycobionts (Molnár and Farkas 2010). Among them a relatively small number of these secondary products (50–60) occur in non-lichenized fungi or higher plants (Elix and Stocker-Wörgötter 2008). One example is the anthraquinone parietina which is present in other fungi like *Aspergillus* and *Penicillium*, as well as in the vascular plant genera *Rheum*, *Rumex*, and *Ventilago* (Romagni and Dayan 2002). This metabolic diversity is largely due to the symbiotic relationship between the lichen partners (Lawrey 1986), and lichen secondary products can comprise up to 20 % of the dry thallus weight, but in most lichens the amount varies from 5 to 10 %. Many lichen secondary metabolites exhibit cytotoxic properties and could be potential sources of pharmaceutically useful chemicals. The purpose of this study was to provide insights regarding the anticancer properties of lichen secondary metabolites and also to provide information regarding the mode of action of lichen compounds against cancer cells. However, so far a limited number of studies were published where the mechanism of action against cancer cell lines had been explored (Molnár and Farkas 2010). The

molecular mechanism of cell death by lichen compounds includes cell cycle arrest, apoptosis, necrosis, and inhibition of angiogenesis (Brisdelli et al. 2013). There is clearly an urgent need for expanding research in this area of study, including studies of those compounds which have shown promising results as well as a strong focus on identifying specific mechanisms of action and extensive clinical trials using the most promising lichen-based drug therapies followed by large-scale production of the best of those compounds. A large number of representatives of this group have already been tested and have been the source of pharmaceutically important anticancer drugs, but there still remains a vast potential reservoir of untapped possibilities. Among the more promising possibilities are lichenized fungi with their more than 1,000 identified secondary chemicals. The use of lichen secondary products as anticancer drugs dates back to the late 1960s when the activity of lichen polysaccharides against tumor cells was initially explored (Fukuoka et al. 1968; Shibata et al. 1968). Similarly, in early studies of Kupchan and Kopperman (1975), they first reported the tumor inhibitor activity of usnic acid extracted from *Cladonia* sp. against Lewis lung carcinoma. They reported a 35–52 % increase in the life span of treated mice versus the control group using a dose range of 20–200 mg/kg of usnic acid. The butyrolactone, protolichesterinic acid, was also found active as an antiproliferative against leukemia cells K-562 (IC50 ¼ 20 mg/ml) and against Ehrlich solid tumor, while nephrosteranic acid derivatives have a poor activity (Hirayama et al. 1980). Polyporic acid (a terphenylquinone) and derivatives (Cain 1966); a depsidone, physodalic acid (Shibamoto and Wei 1984); and lichen glucans (Nishikawa et al. 1969, 1979; Hirayama et al. 1980; Nishikawa and Ohno 1981) including lichenin derivatives (Demleitner et al. 1992) have also been investigated in this way.

5.3 Overview of the Most Investigated Lichen Secondary Metabolites

5.3.1 Usnic Acid

Usnic acid, the most extensively studied lichen metabolite since its first isolation in 1844, exhibited an antiproliferative effect on human leukemia cells (K562) and endometrial carcinoma (HEC-50) cells (Cardarelli et al. 1997; Ingólfsdóttir 2002; Kristmundsdóttir et al. 2002). Therefore, the usnic acid is one of the most interesting lichen metabolites for the study of their antitumor effects. The cytotoxicity, the in vitro antitumor effects, and the mechanism of action of usnic acid need to be investigated in greater detail in order to reach clinical trials and to allow further applications (Table 5.1).

Usnic acid and usnic acid-amine derivatives showed in vitro antiproliferative/cytotoxic activity against a wide variety of murine and human cancer cell lines (Takai et al. 1979; Cardarelli et al. 1997; Bézivin et al. 2004; Mayer et al. 2005;

Table 5.1 Overview of recent literature related to in vitro anticancer activity of lichen secondary metabolites (according to Shrestha and St. Clair 2013)

Lichen metabolites/lichen species	Cell lines tested	References
Usnic acid (commercial) Atranorin (commercial) Parietin (*X. parietina*) Gyrophoric acid (*Umbilicaria hirsuta*)	Human ovarian carcinoma A2780 Human colon adenocarcinoma HT-29	Bačkorová et al. (2012)
Diffractaic acid (*Protousnea magellanica*) Vicanicin (*Psoroma pallidum*) Lobaric acid (*Stereocaulon alpinum*) Variolaric acid (*Ochrolechia deceptionis*) Protolichesterinic acid (*Cornicularia aculeate*) Usnic acid (*Cladonia lepidophora*)	Human breast adenocarcinoma MCF-7 Human colon adenocarcinoma HCT-116 Human cervix adenocarcinoma HeLa	Brisdelli et al. (2012)
Atranorin (*Bacidia stipata*) Diffractaic acid (*P. magellanica*) Divaricatic acid (*Protousnea malacea*) Vicanicin (*Psoroma malacea*) Protolichesterinic acid (*R. melanophthalma*)	Human prostate cancer androgen responsive (LNCaP) Human prostate cancer Androgen nonresponsive DU-145	Russo et al. (2012)
Usnic acid (commercial) Atranorin (commercial) Parietin (*X. parietina*) Gyrophoric acid (*U. hirsuta*)	Human ovarian A2780 Human breast MCF-7 Human colon HT-29 Human T cells, Jurkat Human cervix HeLa Human breast SK-BR-3 Human colon wild-type p53 HCT-116 p53+/+ Human colon p53 null HCT-116 p53−/−	Bačkorová et al. (2012)
Lecanoric acid and its orsellinate derivatives	Larynx carcinoma HEP-2 Breast carcinoma MCF-7 Kidney melanoma cell B16-F10 Vero cell	Bogo et al. (2010)
(+) Usnic acid (*C. arbuscula*) (−) Usnic acid (*Alectoria ochroleuca*)	Breast cancer cell line T-47D Pancreatic cancer cell line Capan-2	Einarsdóttir et al. (2010)
Retigeric acid A and retigeric acid B (*Lobaria kurokawae*)	Human Pca LNCaP PC-3, DU 145 Human epidermoid KB and vincristine Resistant KB (KB/VCR) Human ovarian 3-AO and cisplatin resistant 3-AO (3-AO/CDDP) Human benign prostate Epithelial RWPEI Human hTERT-RPEI Human breast MCF-7	Liu et al. (2010)

(continued)

Table 5.1 (continued)

Lichen metabolites/lichen species	Cell lines tested	References
	Human osteosarcoma U2OS and Saos2	
Olivetoric acid (*Pseudevernia furfuracea*)	Rat adipose tissue endothelial cells	Koparal et al. (2010)
Usnic acid (commercial)	Breast cancer cell lines MCF-7 (estrogen dependent, wild-type p53) Lung cancer cell line H1299 (null for p53)	O'Neill et al. (2010)
(+) Usnic acid (*Xanthoparmelia somloensis*) Salazinic acid (*X. somloensis*) Vulpinic acid (*L. vulpina*) Gyrophoric acid (*Lasallia pustulata*) Evernic acid (*E. prunastri*)	Malignant mesothelioma cells MM98 Vulvar carcinoma cells A431 Keratinocytes HaCaT	Burlando et al. (2009)
16-*O*-Acetyl-leucotylic acid Leucotylic acid (both from *Myelochroa aurulenta*)	Human leukemia cells HL-60	Tokiwano et al. (2009)
Usnic acid and its 9 derivatives	Lymphocytic leukemia L 1210 Murine Lewis lung 3LL Chronic myelogenous leukemia K-562 Brain metastasis of prostate DU 145 Breast MCF 7 Glioblastoma U251 Hamster cell lines: CHO and CHO-MG	Bazin et al. (2008)
Sphaerophorin (*Sphaerophorus globosus*) Pannarin (*Psoroma* spp.)	Human melanoma cells M14	Russo et al. (2008)
(+) Usnic acid (*R. farinacea*) (−) Usnic acid (*Cladonia foliacea*)	Chinese hamster lung fibroblast V79 Human lung V79 Human lung carcinoma A549	Koparal et al. (2006)
Sphaerophorin (*S. globosus*) Pannarin (*Psoroma* spp.) Epiphorellic acid-1 (*Cornicularia epiphorella*)	Human prostrate carcinoma DU 145 Normal human prostatic epithelial cells	Russo et al. (2006)
Usnic acid (commercial)	Breast cancer MCF-7 (estrogen dependent, wild-type p53) Breast cancer cell lines MDA-MB-231 (estrogen independent, mutant p53) Lung cancer cell line H1299	Mayer et al. (2005)
(−) Usnic acid Fumarprotocetraic acid 90-(*O*-methyl) protocetraric acid	Murine leukemia L1210 Murine Lewis lung 3LL Chronic myelogenous leukemia U215	Bézivin et al. (2004)

(continued)

Table 5.1 (continued)

Lichen metabolites/lichen species	Cell lines tested	References
	Human brain metastasis of a prostate DU 145 Human breast MCF 7 Human glioblastoma RCB-0461	
Depsidones—vicanicin, pannarin, 1-chloropannarin, salazinic acid, stictic acid, variolaric acid, psoromic acid, fumarprotocetraric acid, lobaric acid Depsides—atranorin, sphaerophorin, divaricatic acid, diffractaic acid gyrophoric acid, usnic acid	Hepatocytes from rat	Correché et al. (2004)
Pannarin, 10 chloropannarin Salazinic acid, psoromic acid Fumarprotocetraic acid, lobaric acid Vicanicin, stictic acid Variolaric acid, atranorin Sphaerophorin, divaricatic acid Diffractaic acid, gyrophoric acid	Lymphocytes from rat spleens	Correché et al. (2002)
(+) Usnic acid Methyl a-orcinolcarboxylate Ethyl hematommate Diffractaic acid (+) Protolichesterinic acid	Human keratinocyte cell line HaCaT	Kumar and Muller (1999)
Lobaric acid (*S. alpinum*) Protolichesterinic acid (*C. islandica*)	Breast cancer cell T-47D and ZR-75-1 Erythroleukemia K-563	Ogmundsdottir et al. (1998)
Usnic acid derivatives	Lewis lung carcinoma L1210	Takai et al. (1979)

Bazin et al. 2008; Sahu et al. 2011; Bačkorová et al. 2011; Burlando et al. 2009). The toxicity of usnic acid was associated with increased P450 activity and oxidative stress in human hepatoblastoma cells (Sahu et al. 2011); with mitochondrial dysfunction in HepG2 cells (Sahu et al. 2011), in the breast cancer T-47D cell line and in the pancreatic cancer Capan-2 cell line (Einarsdóttir et al. 2010); and with apoptotic induction in murine leukemia L1210 cells (Bézivin et al. 2004; Bazin et al. 2008).

(+) Usnic acid was found to be a strong hepatotoxic agent against monogastric murine hepatocytes (Han et al. 2004). Also, Correché et al. (2004) investigated the cytotoxic and apoptotic effects of usnic acid obtained from Continental (Chilean) and Antarctic lichens in primary cultures of rat hepatocytes.

The (−) enantiomer of usnic acid (isolated from *Cladonia convoluta*) was moderately cytotoxic to various cancer cell lines, such as murine Lewis lung carcinoma, human chronic myelogenous leukemia, human brain metastasis of a prostate carcinoma, human breast adenocarcinoma, and human glioblastoma (Molnár and Farkas 2010: Bézivin et al. 2004). Usnic acid also decreased

proliferation of human breast cancer cells and human lung cancer cells without any DNA damage (Mayer et al. 2005). Finding cancer therapies that do not have DNA-damaging effects and that do not cause the development of secondary malignancies later in life is of great interest. Accordingly, usnic acid may represent a novel source for a natural non-genotoxic anticancer drug. Usnic acid from the lichen *Usnea barbata* (Rankovic et al. 2012) induced a significant cytotoxic effect on the tested human melanoma Fem-x and human colon carcinoma LS174 cell lines, which was stronger than the lichen extracts. Then, as shown by numerous data, there is a significant antitumor activity of usnic acid in vitro. Here are some of them. Usnic acid activated programmed cell death in A2780 and HT-29, probably through the mitochondrial pathway (Bačkorová et al. 2012).

Lichen compounds showed differential sensitivity to various cancer cells. Usnic acid was highly effective against the whole spectrum of cell lines (HeLa, MCT-7, A2780, HT-29, Jurkat, SK-BR-3, and HCT-116). Similar to cytotoxicity, usnic acid also significantly inhibited the clonogenic ability of all the tested cell lines. Also, usnic acid demonstrated strong pro-apoptotic action associated with the altered cell cycle distribution and accumulation of cells in S phase (Bačkorová et al. 2011).

Somewhat earlier (Einarsdóttir et al. 2010) it was announced that both (+) and (−) usnic acids are effective inhibitors of DNA synthesis, with IC50 values of 4.2 and 4.0 μg/ml against T-47D (breast cancer cell line) and 5.3 and 5.0 μg/ml against Capan-2 (pancreatic cancer cell line). There was a reduction in cell size and both acids inhibit cell entry into the S phase. Regarding the mechanism of action, staining with the mitochondrial dye JC-1 demonstrated a dose-dependent loss of mitochondrial membrane potential following treatment with usnic acid in both cell lines. A study on the effects of usnic acid on MCF-7 (estrogen dependent, wild-type p53) indicated no morphological changes in microtubules or increase in the mitotic index. This suggests that the antineoplastic activity of usnic acid is not related to alterations in the formation and/or stabilization of microtubules (O'Neill et al. 2010).

In usnic acid from *Xanthoparmelia somloensis*, Burlando et al. (2009) have investigated its cytotoxic effect towards malignant mesothelioma cells (MM98), vulvar carcinoma cells (A431), and keratinocytes (HaCaT). Usnic acid showed high cytotoxicity for all three cell lines. Further, both types of usnic acid showed dose- and time-dependent cytotoxicity against V79 (Chinese hamster lung fibroblast) and A549 (human lung carcinoma) cell lines. Cytotoxicity was more pronounced in A549 than V79 with cell viability more diminished in A549 versus V79 after 2 days of treatment (Koparal et al. 2006). In order to investigate the mechanism of action of usnic acid, elevated levels of the p53 and p21 proteins were confirmed following treatment with usnic acid, but there was no p53 transcriptional activity, suggesting that the accumulation of p21 was not secondary to p53 transactivation (Mayer et al. 2005). They concluded that usnic acid has antiproliferative activity against wild-type p53 (MCF-7) and nonfunctional p53 (MDA-MB-231) breast cancer cells, as well as against the H1299 lung cancer cell line, which is null for p53. Usnic acid is therefore a non-genotoxic anticancer agent that works in p53-independent manner. In another study (Bézivin et al. 2004), usnic acid also induced L1210 (murine

lymphocytic leukemia) in apoptosis in a dose- and time-dependent manner as fluorescence microscopy revealed condensation of nuclear chromatin, nuclear fragmentation, and formation of apoptotic bodies.

5.3.2 Depsides and Depsidones

There are several studies about antitumor activity of depsides and depsidones and especially on atranorin. Bačkorová and colleagues reported that antiproliferative/cytotoxic effects of atranorin efficiently induced apoptosis and inhibited cell proliferation in various cancer cell lines tested. Similar with usnic acid, atranorin demonstrated strong pro-apoptotic action. Moreover, the same authors reported on the sensitivity of up to nine human cancer cell lines (A2780, HeLa, MCF-7, SK-BR-3, HT-29, HCT-116, p53 (+/+), HCT-116, p53 (−/−), HL-60, and Jurkat) to the antiproliferative/cytotoxic effects of same typical secondary metabolites of lichens (parietin and gyrophoric acid). Further, the analysis of cell cycle distribution also revealed an accumulation of cells in S phase. This study has confirmed a differential sensitivity of cancer cell lines to lichen secondary metabolites (Bačkorová et al. 2011). In addition, atranorin, diffractaic acid, and divaricatic acid were found to be active against prostate cancer cells (human prostate cancer androgen-responsive (LNCaP) and human prostate cancer androgen-nonresponsive DU-145 cells) only in high concentration (Russo et al. 2006, 2012). This study for the first time showed that apoptosis induced by the compounds appeared to be mediated, at least in part, via the inhibition of Hsp70 expression. Also, depsides—atranorin, sphaerophorin, divaricatic acid, diffractaic acid, and gyrophoric acid—and depsidones, vicanicin, pannarin, 1-′chloropannarin, salazinic acid, stictic acid, variolaric acid, psoromic acid, fumarprotocetraric acid, and lobaric acid, were evaluated for their cytotoxic activity towards hepatocytes from rat and lymphocytes from rat spleens (Correché et al. 2002, 2004). The research has shown that salazinic acid, stictic acid, and psoromic acid showed apoptosis of hepatocytes in a dose-dependent manner with stictic acid showing the strongest apoptotic activity. Ogmundsdottir and associates show that lobaric acid and protolichesterinic acid towards breast cancer cells T-47D and ZR-75-1 as erythroleukemia K-563 cells caused a significant reduction in DNA synthesis. Significant cell deaths in three malignant cell lines (T-47D and ZR-75-1 from breast carcinomas and K-562 from erythro-leukaemia), were observed at concentrations of 20 and 30 μg/ml of protolichesterinic acid and lobaric acid, respectively (Ogmundsdóttir et al. 1998). Similarly, gyrophoric acid, usnic acid, and diffractaic acids were reported as potent antiproliferative agents which inhibited cell growth at IC_{50} values of 1.7, 2.1, and 2.6 μM on human keratinocyte cell line HaCaT (Kumar and Muller 1999). Also, in the work of Pejin and associates, it is shown that the results suggest a moderate anticancer activity towards malignant HT-29 (IC50 value was 29.29 μg/ml) and a low growth inhibition on nonmalignant MRC5 cells (IC50 value was 2,478.40 μg/ml) of stictic acid (Pejin et al. 2013). This may indicate that stictic acid can be

considered as a promising lead compound for the design of novel human colon adenocarcinoma drugs. Generally, depsidones showed stronger cytotoxic activity than depsides. The strong biological activity of some depsidones may be due to the strong hydrogen bond between the aldehyde group at C3 and the hydroxyl group at C4. Similarly, the cytotoxic activity of depsides may be in part due to the presence of a COOH group on C′1 and an OH group on C′2. Likewise, Manojlovic and associates reported the strong cytotoxic effect of depsidone salazinic acid as well as phenolic compound protocetraric acid against Fem-x (human melanoma) and LS174 (human colon carcinoma) cell lines (Manojlovic et al. 2012). Pannarin, a depsidone, was shown to inhibit growth of DU-145 prostate carcinoma and M14 human melanoma cells (Russo et al. 2006; Brandão et al. 2013) Also, in the purpose of identifying novel agents with antigrowth and pro-apoptotic activity on prostate cancer cells, Russo et al. (2012) evaluated the effect of lichen secondary metabolites; the depsides atranorin, diffractaic, and divaricatic acids; as well as the depsidone vicanicin on cell growth in the androgen-sensitive (LNCaP) and androgen-insensitive (DU 145) human prostate cancer cells. The depsides resulting from decarboxylation of baeomycesic and squamatic acids showed antiproliferative effects on PC-3 prostate cancer cells (50 % growth inhibitory concentration (GI50) 70.06, 79.37 μm, respectively) (Guo et al. 2011). Also, olivetoric acid as di-depside displayed dose-dependent antiangiogenic activities, inhibited cell proliferation, and disrupted endothelial tube formation in rat adipose tissue endothelial cells (Koparal et al. 2010).

Very good cytotoxic activity against malignant Fem-x and LS174 cells also showed depsidone physodic acids and depside atranorin, which were identified from the lichen *H. physodes* growing in Serbia (Rankovic et al. 2014). Their IC_{50} values were in the range of 17.89 to 24.63 μg/ml, respectively, something less than the value of the IC_{50} of usnic acid. These authors examined the antitumor activity of evernic acid, which belongs to depsides, and also depsidone physodic acid isolated from the lichens *E. prunastri* and *P. furfuraceae* (Kosanic et al. 2013). The obtained results show that the tested compounds exhibited high cytotoxic activity against the target cells in vitro. The best cytotoxic activity was exhibited the physodic acid. The effect of tested samples on cell cycle progression was investigated also in Fem-x and LS174 cells. An increase in cells containing sub-G1 amounts of DNA was observed, indicating that the evernic and physodic acids were inducing cell death. Similarly, somewhat earlier Russo et al. (2008) reported that the depside sphaerophorin (isolated from *Sphaerophorus globosus*) and the depsidone pannarin (isolated from *Psoroma pholidotoides*) inhibited the growth of M14 human melanoma cells, triggering apoptotic cell death. The data obtained from cell culture show that these lichen metabolites inhibit the growth of melanoma cells, inducing their apoptotic cell death, demonstrated by the fragmentation of genomic DNA and by a significant increase of caspase-3 activity and correlated, at least in part, to the increase of ROS generation. The anticancer activities of these lichen metabolites are promising in the treatment of this aggressive, therapy-resistant skin tumor (Molnár and Farkas 2010). However, it is done in one new study (Brisdelli et al. 2013) of six lichen metabolites (diffractaic acid, lobaric acid, lips acid,

vicanicin, variolaric acid, protolichesterinic acid) wherein its effects on proliferation, viability, and reactive oxygen species (ROS) level towards three human cancer cell lines MCF-7 (breast adenocarcinoma), HeLa (cervix adenocarcinoma), and HCT-116 (colon carcinoma) were investigated. In this comparative study, lichen metabolites showed various cytotoxic effects in a concentration-dependent manner. Moreover, all tested lichen compounds did not exhibit free radical scavenging activity. The lichen metabolites did not significantly increase the intracellular ROS level. Further, the cytotoxic activities of depsidone, variolaric acid, and two other secondary metabolites of lichens, R-alectoronic acid and ergosterol peroxide, were evaluated against the murine B16 melanoma cell line. All the tested compounds showed a significant antitumor activity, especially variolaric acid and alectoronic acid, as compared to cisplatin as a positive control (Millot et al. 2007). Anziaic acid was also found to act as an inhibitor of human topoisomerase II but had little effect on human topoisomerase I (Cheng et al. 2013). This is the first report of a depside with activity as a topoisomerase poison inhibitor and demonstrates the potential of this class of natural products as a source for new antibacterial and anticancer compounds. Protolichesterinic acid showed an inhibitory effect against 12 cell lines, with IC_{50} values of 2.4–18.1 μg/mL (Russo et al. 2012). Also, in recent years (Bogo et al. 2010), it has been shown that lecanoric acid, (para-depside) a secondary metabolite of the lichen *Parmotrema tinctorum*, has moderate antitumor activity against some malignant cell lines (MCF-7 breast carcinoma, 786-0 kidney carcinoma, and B16-F10 murine melanoma) tested. Similarly, some cytotoxic activity in vitro of lecanoric acid and orsellinic acid methyl ester, orcinol, and usnic acid isolated from the lichen *Parmelia subrudecta* is shown by other authors (Ivanova et al. 2010). The depsides resulting from decarboxylation of baeomycesic and squamatic acids showed antiproliferative effects on PC-3 prostate cancer cells (Haraldsdóttir et al. 2004; Brandão et al. 2013). Also, Brandao and his research group have reported the results of the evaluation of some lichen compounds (depsides atranorin and diffractaic, divaricatic, and perlatolic acids; the depsidones psoromic, protocetraric, and norstictic acids) tested against UACC-62 and B16-F10 melanoma cell lines and 3T3 normal fibroblast cells (Brandão et al. 2013).

5.3.3 Naphthoquinones

Naphthazarin and its derivates were isolated from *Cetraria islandica*. This naphthoquinone demonstrates in in vitro experiments strong cytotoxic effect to human epidermal carcinoma cells. Dimer of this naphthoquinone, hybocarpone, was isolated from *Lecanora hybocarpa* (Babula et al. 2009).

5.3.4 Anthraquinones

Emodin
Anthraquinones represent a large family of compounds having diverse biological properties. Emodin (1,3,8-trihydroxy-6-methylanthraquinone) is a naturally occurring anthraquinone present in the numerous lichens (Cohen and Towers 1995). Emodin, first assigned to be a specific inhibitor of the protein tyrosine kinase p65lck, has now a number of cellular targets interacting with it. Its inhibitory effect on mammalian cell cycle modulation in specific oncogene overexpressed cells formed the basis of using this compound as an anticancer agent. Identification of apoptosis as a mechanism of elimination of cells treated with cytotoxic agents initiated new studies deciphering the mechanism of apoptosis induced by emodin. At present, its role in combination chemotherapy with standard drugs to reduce toxicity and to enhance efficacy is pursued vigorously. Its additional inhibitory effects on angiogenic and metastasis regulatory processes make emodin a sensible candidate as a specific blocker of tumor-associated events (Srinivas et al. 2007).

Parietin
Parietin is derived from polyaromatic ring polyketides and is present in lichen of genera *Xanthoria* and *Teloschistes* in particular in the lichens *Teloschistes chrysophthalmus*, *Teloschistes spinosus*, and *Xanthoria parietina*. Among others, Bačkorová and the authors showed the certain cytotoxic potential of parietin by a series of cancer cell lines (Bačkorová et al. 2011).

5.3.5 Xanthones: Lichexanthone

Lichexanthone is one of the xanthones tested also in a recent study (Brandão et al. 2013) together with other secondary metabolites of lichens. This study revealed the following: lichexanthone was the least active substance tested, delineating a very distinct response relative to the other compounds and the standard doxorubicin.

5.3.6 Others: Some Specific Class of Compounds

Apart from compounds derived from common pathways, which are found throughout all major lichen groups, there are also some unusual compound classes among these organisms; for example, arthogalin, a cyclic depsipeptide (Huneck and Himmelreich 1995), and other amino acid-derived compounds such as the cytotoxic scabrosin esters isolated from *Xanthoparmelia scabrosa* (Ernst-Russell et al. 1999). Thus, Magaya and colleagues test the effects of arthogalin, a secondary metabolite of the lichen *Caloplaca inclinans*, on the growth of murine malignant prostate

sarcoma cells in vitro (Magaya et al. 2013). The results of this study showed that arthogalin is a potent inhibitor of growth of tested cancer cells. They also showed that arthogalin increases sensitivity of cells to radiation, and this effect is significant at a radiation intensity lower than the standard intensity of cancer radiotherapy. On the basis of this study, arthogalin shows promise for combined-modality cancer treatment. Also, earlier studies have shown that scabrosin esters (SEs), which have been isolated from the lichen *Xanthoparmelia scabrosa*, belong to the epipolythiodioxopiperazine (ETP) class of secondary metabolites characterized by possession of a reactive disulfide bond. Colony-forming assays, which were used in these studies, have shown that these compounds are active against human tumor cell lines at nanomolar concentrations. Colony-forming assays show that these toxins are active against human tumor cell lines at nanomolar concentrations (Moerman et al. 2003). These authors show that the typical scabrosin ester acetate butyrate induces early mitochondrial membrane hyperpolarization accompanied by apoptotic cell death. Here we will mention retigeric acid A (RA) and retigeric acid (RB), both a pentacyclic triterpenoids from the lichen species *Lobaria kurokawae*. Liu et al. (2010) showed cytotoxicity towards malignant cells at lower concentrations (>100 μM) of these compounds, but RB is more potent than RA. Specially, investigation on the effect of RB on PC-3 cells showed that RB caused a dose-dependent accumulation of cells in the S phase accompanied with decreases in cyclin B and increases in cyclin E and cyclin A. Both caspase-dependent and caspase-independent pathways were responsible for apoptosis in PC-3 cells. It should also be noted that 16-*O*-acetyl-leucotylic acid, a new triterpenic acid, exhibited potent antiproliferative activity against HL-60 with an EC50 value of 21 μM, while the leucotylic acid, derivative of 16-*O*-acetyl-leucotylic acid, has a higher EC50 value (72 μM) (Tokiwano et al. 2009).

5.4 Overview of Existing In Vivo Studies

Therefore, without any in vivo evaluation and clinical trials, no real efficacy in cancer therapy can be argued for any of the potential agents. Firstly, it is known that most of the antitumor in vivo studies on lichen extracts have been performed by Japanese scientists in the 1970s (Fujikawa et al. 1972; Hirayama et al. 1974). The allogeneic tumor S-180-forming ascites implanted into albino mice is generally used as the basic screening model. Few antitumor assays have been conducted on Ehrlich carcinomas. In each model, samples dissolved in distilled water were administered by i.p. injection for 10 consecutive days, starting 24 h after tumor implantation. After 30 days, the antitumor effect was evaluated through the inhibition ratio (IR), which is linked to the reduction of tumor weight, and the complete regression rate (CR), which is linked to complete recovery of grafted animals. However, with advances in the isolation and characterization of secondary metabolites of lichens, the growing interest for the in vitro testing of their antitumor effects, and in vivo studies. (Ribeiro-Costa et al. 2004). Only scarce in vivo assays

in mice have been attempted for some low molecular weight lichen compounds. One of the first lichen acids found with some activity on L-1210 and S-180 models in mice was polyporic acid, a dihydroxyquinone isolated from *Sticta coronata*. It was given in a dose of 60 mg/kg administered by intraperitoneal (i.p.) injection (Burton and Cain 1959). The well-known (−) usnic acid, a dibenzofuran, was proved to have a weak, if any, antitumoral effect against Lewis lung carcinoma and P-388 leukemia cells (Kupchan et al. 1975 cited in Takai et al. 1979). A series of 20 lichen compounds have been tested against Ehrlich carcinomas in mice, revealing some potential for the butyrolactones (+) protolichesterinic acid and nephrosterinic acid (50 and 70 % tumor growth inhibition, respectively) (Hirayama et al. 1980). A significant in vivo antineoplastic activity (murine leukemia P-388, tested/control × 100 (T/C) = 40 % at 160 μg/kg) is reported for ambewelamide A, an original diketopiperazine dione (Williams et al. 1998). This scabrosin ester and derivatives isolated from two lichen species have shown potent in vitro cytotoxic activities (IC_{50} within the μM to nM range for P = 388, P-815 and MCF-7) (Williams et al. 1998; Ernst-Russell et al. 1999). Another rare compound, hybocarpone isolated from a mycobiont culture of *Lecanora hybocarpa* (IC_{50} = 0.27 μM) (Ernst-Russell et al. 1999), is also a relevant compound to be investigated further in terms of anticancer studies. In 2004 R. M. Ribeiro-Costa and colleagues investigated the in vitro and in vivo properties of usnic acid encapsulated into PLGA microspheres. Microparticles will probably play a promising role in the future of chemotherapy. These polymeric delivery systems are capable of maximizing the therapeutic activity while reducing side effects of anticancer agents. In this study, poly(lactic-*co*-glycolic acid) (PLGA) microspheres contain a usnic acid from *Cladonia substellata*. The antitumor assay was performed in mice against sarcoma-180 tumor (UA 15 mg/kg weight body/day) during 7 days. Animals were then sacrificed, and tumor and organs were excised for histopathological analysis. A maximum release of 92 % was achieved at the fifth day. The IC50 values for free and encapsulated usnic acid were 12 and 14 μg/ml, respectively. The encapsulation of usnic acid into microspheres promoted an increase of 21 % in the tumor inhibition as compared with the free usnic acid treatment. In summary, usnic acid was efficiently encapsulated into PLGA microspheres and the microencapsulation improved its antitumor activity (Ribeiro-Costa et al. 2004).

5.5 Lichen Secondary Metabolites as Potential Anticancer Drugs: Prospects and Promise

Chemotherapy is still the method most commonly used and most promising in the treatment of cancer patients. Also, chemotherapy holds the most promise for selectively eradicating cancer cells while at the same time minimizing collateral damage to surrounding tissues. Many of these chemical agents owe their origins to natural sources in the environment, whereas other anticancer chemotherapeutics are

wholly designed by pharmaceutical scientists based upon current knowledge of cancer onset mechanisms. Selectivity for cancer cell destruction without harming healthy cells is the central focus of these treatment protocols, and chemotherapy's well-known side effects (hair loss, nausea, immunodeficiency, etc.) are a continuing reminder that much room for progress remains. Natural products and their derivatives represent more than 50 % of all the drugs in clinical use of the world. Almost 60 % of drugs approved for cancer treatment are of natural origin (Fakim 2006). Whether the promise of fully selective anticancer medicines will be realized in our lifetime remains unknown, but exciting developments from the investigation of lichen secondary metabolites lend credibility to the proposition that the best is yet to come. Therefore, increasing research on lichen natural resource may provide good results for exploiting and developing valuable natural products which benefit for human. For the past 20–30 years, some studies with lichen, even with the limited screening effort, have indicated the frequent occurrence of metabolites with antitumor properties (Ren et al. 2009; Rankovic et al. 2011; Kosanic et al. 2012a, b; Rankovic et al. 2014). All of this supports efforts to the development of new anticancer drugs that have as a starting point lichen secondary metabolites. The potential of lichen secondary metabolites as a possible source of anticancer drugs is certainly large and visible. As we know, the structure of more than 700 lichen substances is available, but due to the slow growth of lichen, their availability is insufficient in quantity and has difficulty in large-scale industrial production; lichens were frequently ignored by pharmaceutical industries. However, the secondary metabolites of lichen which are deposited on the surface of mycelium were usually produced by fungi; therefore, it becomes possible that cultured mycobionts could replace the lichen. Although many of lichen metabolites are not likely to become therapeutics, the information gained from studying them is likely to lead to the development and understanding of novel molecular targets and chemical synthesis or chemical modification of natural metabolites, which in turn may lead to the development of new classes of therapeutic agents. On the other hand, powerful new technologies such as combinatorial chemistry, high-throughput screening, bioinformatics, proteomics, and genomics have emerged and are being integrated widely in the field of pharmaceutical discovery research. These technologies have enormous potential to make use of the chemical diversity of natural products (Lahlou 2013). All this, including compound library design, protein 3D structures, NMR-based screening, 3D QSAR in modern drug design, and computer-aided prediction of drug toxicity and metabolism, may help in the development of new agents modeled on the basis of secondary metabolites of lichens. Finally, a multidisciplinary collaboration among lichenologists, chemists, pharmacologists, and biologists should be crucial in the development of potential anticancer drugs from secondary metabolites of lichens.

References

Babula P, Adam V, Havel L et al (2009) Noteworthy secondary metabolites naphthoquinones—their occurrence, pharmacological properties and analysis. Curr Pharm Anal 5:47–68

Bačkorová M, Bačkor M, Mikeš J et al (2011) Variable responses of different human cancer cells to the lichen compounds parietin, atranorin, usnic acid and gyrophoric acid. Toxicol In Vitro 25:37–44

Bačkorová M, Jendželovský R, Kello M et al (2012) Lichen secondary metabolites are responsible for induction of apoptosis in HT-29 and A2780 human cancer cell lines. Toxicol In Vitro 26: 462–468

Bazin MA, Le Lamer AC, Delcros JG et al (2008) Synthesis and cytotoxic activities of usnic acid derivatives. Bioorg Med Chem 16:6860–6866

Behera BC, Adawadkar B, Makhija U (2003) Inhibitory activity of xanthine oxidase and superoxide-scavenging activity in some taxa of the lichen family Graphidaceae. Phytomedicine 10:536–543

Behera BC, Adawadkar B, Makhija U (2004) Capacity of some Graphidaceous lichens to scavenge superoxide and inhibition of tyrosinase and xanthine oxidase activities. Curr Sci 87:83–87

Bézivin C, Tomasi S, Rouaud I et al (2004) Cytotoxic activity of compounds from the lichen: Cladonia convoluta. Planta Med 70:874–877

Bogo D, Matos MFC, Honda NK et al (2010) In vitro antitumor activity of orsellinates. Z Naturforsch 65:43–48

Boustie J, Grube M (2005) Lichens—a promising source of bioactive secondary metabolites. Plant Genet Resour 3:273–287

Brandão LF, Alcantara GB, Matos Mde F et al (2013) Cytotoxic evaluation of phenolic compounds from lichens against melanoma cells. Chem Pharm Bull (Tokyo) 61:176–183

Brisdelli F, Perilli M, Sellitri D et al (2013) Cytotoxic activity and antioxidant capacity of purified lichen metabolites: an in vitro study. Phytother Res 27:431–437

Brunauer G, Hager A, Krautgartner WD et al (2006) Experimental studies on *Lecanora rupicola* (L.) Zahlbr.: chemical and microscopical investigations of the mycobiont and re-synthesis stages. Lichenologist 38:577–585

Brunauer G, Hager A, Grube M et al (2007) Alterations in secondary metabolism of aposymbiotically grown mycobionts of *Xanthoria elegans* and cultured resynthesis stages. Plant Physiol Biochem 45:146–151

Burlando B, Ranzato E, Volante A et al (2009) Antiproliferative effects on tumour cells and promotion of keratinocyte wound healing by different lichen compounds. Planta Med 75: 607–613

Burton JF, Cain BF (1959) Antileukæmic activity of polyporic acid. Nature 184:1326–1327

Cain BF (1966) Potential anti-tumour agents. IV. Polyporic acid series. J Chem Soc Perkin 11: 1041–1045

Cardarelli M, Serino G, Campanella L et al (1997) Antimitotic effects of usnic acid on different biological systems. Cell Mol Life Sci 53:667–672

Cheng B, Cao S, Vasquez V et al (2013) Identification of anziaic acid, a lichen depside from Hypotrachyna sp., as a new topoisomerase poison inhibitor. PLoS One 8(4):e60770

Cohen PA, Towers GHN (1995) Anthraquinones and phenanthroperylenequinones from Nephroma laevigatum. J Nat Prod 58:520–526

Correché ER, Carrasco M, Giannini F et al (2002) Cytotoxic screening activity of secondary lichen metabolites. Acta Farm Bonaerense 21:273–278

Correché ER, Enriz RD, Piovano M et al (2004) Cytotoxic and apoptotic effects on hepatocytes of secondary metabolites obtained from lichens. Altern Lab Anim 32:605–615

Crittenden D, Porter N (1991) Lichen-forming fungi: potential sources of novel metabolites. Trends Biotechnol 9:409–414

Demleitner S, Kraus J, Franz G (1992) Synthesis and antitumour activity of derivatives of curdlan and lichenan branched at C-6. Carbohydr Res 226:239–246

Einarsdóttir E, Groeneweg J, Björnsdóttir GG et al (2010) Cellular mechanisms of the anticancer effects of the lichen compound usnic acid. Planta Med 76:969–974

Elix JA (1996) Biochemistry and secondary metabolites. In: Nash TH III (ed) Lichen biology, 1st edn. Cambridge University Press, Cambridge, pp 155–180

Elix JA, Stocker-Wörgötter E (2008) Biochemistry and secondary metabolites. In: Nash TH III (ed) Lichen biology, 2nd edn. Cambridge University Press, Cambridge, pp 104–133

Ernst-Russell MA, Chai CLL, Hurne AM et al (1999) Revision and cytotoxic activity of the scabrosin esters, epidithiopiperazinediones from the Lichen *Xanthoparmelia scabrosa*. Aust J Chem 52:279–283

Fahselt D (1994) Secondary biochemistry of lichens. Symbiosis 16:117–165

Fakim AG (2006) Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med 27:1–93

Fujikawa F, Hirai K, Hirayama T et al (1972) Influence of cytochalasin B on hyphal morphogenesis of Aspergillus nidulans. Yakugaku Zasshi 92:823–827

Fukuoka F, Nakanishi M, Shibata S et al (1968) Polysaccharides in lichens and fungi. II. Antitumor activities on sarcoma-180 of the polysaccharide preparations from *Gyrophora esculenta* Miyoshi, *Cetraria islandica* (L.) Ach. var. *orientalis* Asahina, and some other lichens. Gann 59:421–432

Guo J, Li ZL, Wang AL et al (2011) Three new phenolic compounds from the lichen Thamnolia vermicularis and their antiproliferative effects in prostate cancer cells. Planta Med 77: 2042–2046

Hager A, Brunauer G, Türk R et al (2008) Production and bioactivity of common lichen metabolites as exemplified by Heterodea muelleri (Hampe) Nyl. J Chem Ecol 34:113–120

Han D, Matsumaru K, Rettori D et al (2004) Usnic acid-induced necrosis of cultured mouse hepatocytes: inhibition of mitochondrial function and oxidative stress. Biochem Pharmacol 67:439–451

Haraldsdóttir S, Guðlaugsdóttir E, Ingólfsdóttir K et al (2004) Anti-proliferative effects of lichen-derived lipoxygenase inhibitors on twelve human cancer cell lines of different tissue origin in vitro. Planta Med 70:1098–1100

Hirayama T, Fujikawa F, Yosioka I et al (1974) A new depsidone oxyphysodic acid isolated from a lichen Parmelia enteromorphia Ach. Chem Pharm Bull (Tokyo) 22:1678–1680

Hirayama T, Fujikawa F, Kasahara T et al (1980) Anti-tumor activities of some lichen products and their degradation products. Yakugaku Zasshi 100:755–759

Huneck S (1999) The significance of lichens and their metabolites. Naturwissenschaften 86: 559–570

Huneck S, Himmelreich U (1995) Arthogalin, a cyclic depsipeptide from the lichen *Arthothelium galapagoense*. Z Naturforsch 50:1101–1103

Ingólfsdóttir K (2002) Usnic acid. Phytochemistry 61:729–736

Ivanova V, Backor M, Dahse HM et al (2010) Molecular structural studies of lichen substances with antimicrobial, antiproliferative, and cytotoxic effects from Parmelia subrudecta. Prep Biochem Biotechnol 40:377–388

Johnson CJ, Bennett JP, Biro SM et al (2011) Degradation of the disease-associated prion protein by a serine protease from lichens. PLoS One 6:19836–19837

Karikas GA (2010) Anticancer and chemopreventing natural products: some biochemical and therapeutic aspects. J BUON 15:627–638

Koparal AT, Tuylu BA, Turk H (2006) In vitro cytotoxic activities of (+)-usnic acid and (-)-usnic acid on V79, A549, and human lymphocyte cells and their nongenotoxicity on human lymphocytes. Nat Prod Res 20:1300–13007

Koparal AT, Ulus G, Zeytinoglu M et al (2010) Angiogenesis inhibition by a lichen compound olivetoric acid. Phytother Res 24:754–758

Kosanic M, Rankovic B, Stanojkovic T (2012a) Antioxidant, antimicrobial, and anticancer activity of 3 Umbilicaria species. J Food Sci 77:20–25

Kosanic MM, Rankovic BR, Stanojkovic TP (2012b) Antioxidant, antimicrobial and anticancer activities of three Parmelia species. J Sci Food Agric 92:1909–1916

Kosanic M, Manojlovic N, Jankovic S et al (2013) *Evernia prunastri* and *Pseudoevernia furfuraceae* lichens and their major metabolites as antioxidant, antimicrobial and anticancer agents. Food Chem Toxicol 53:112–118

Kristmundsdóttir T, Aradóttir HA, Ingólfsdóttir K et al (2002) Solubilization of the lichen metabolite (+)-usnic acid for testing in tissue culture. J Pharm Pharmacol 54:1447–1452

Kumar KCS, Muller K (1999) Lichen metabolites. 2. Antiproliferative and cytotoxic activity of gyrophoric, usnic, and diffractaic acid on human keratinocyte growth. J Nat Prod 62:821–823

Kupchan SM, Kopperman HL (1975) l-usnic acid: tumor inhibitor isolated from lichens. Experientia 31:625

Lahlou M (2013) The success of natural products in drug discovery. Pharmacol Pharm 4:17–31

Lawrey JD (1986) Biological role of lichen substances. Bryologist 89:111–122

Liu H, Liu Y, Liu Y et al (2010) A novel anticancer agent, retigeric acid B, displays proliferation inhibition, S phase arrest and apoptosis activation in human prostate cancer cells. Chem Biol Interact 188:598–606

Magaya MC, Yeang DP, Ahkeboyee BGW (2013) Arthogalin inhibits the growth of murine malignant prostate sarcoma cells in vitro. J Pharm Pharmacol Res 3:22–26

Manojlovic N, Rankovic B, Kosanic M et al (2012) Chemical composition of three Parmelia lichens and antioxidant, antimicrobial and cytotoxic activities of some their major metabolites. Phytomedicine 19:1166–1172

Mayer M, O'Neill MA, Murray KE et al (2005) Usnic acid: a non-genotoxic compound with anti-cancer properties. Anticancer Drugs 16:805–809

Miao V, Coeffet M, Brown D et al (2001) Genetic approaches to harvesting lichen products. Trends Biotechnol 19:349–355

Millot M, Tomasi S, Articus K et al (2007) Metabolites from the lichen Ochrolechia parella growing under two different heliotropic conditions. J Nat Prod 70:316–318

Moerman KL, Chai CL, Waring P (2003) Evidence that the lichen-derived scabrosin esters target mitochondrial ATP synthase in P388D1 cells. Toxicol Appl Pharmacol 190:232–240

Molnár K, Farkas E (2010) Current results on biological activities of lichen secondary metabolites: a review. Z Naturforsch 65:157–173

Muggia L, Schmitt I, Grube M (2009) Lichens as treasure chests of natural products. SIM News 85–97

Müller K (2001) Pharmaceutically relevant metabolites from lichens. Appl Microbiol Biotechnol 56:9–16

Nishikawa Y, Ohno H (1981) Studies on the water-soluble constituents of lichens. IV. Effect of antitumor lichen-glucans and related derivatives on the phagocytic activity of the reticulo-endothelial system in mice. Chem Pharm Bull (Tokyo) 29:3407–3410

Nishikawa Y, Takeda T, Shibata S et al (1969) Polysaccharides in lichens and fungi. 3. Further investigation on the structures and the antitumor activity of the polysaccharides from Gyrophora esculenta Miyoshi and Lasallia papulosa Llano. Chem Pharm Bull (Tokyo) 17:1910–1916

Nishikawa Y, Yoshimoto K, Horiuchi R et al (1979) Studies on the water-soluble constituents of lichens. III. Changes in antitumor effect caused by modifications of pustulan- and lichenan-type glucans. Chem Pharm Bull (Tokyo) 27:2065–2072

O'Neill MA, Mayer M, Murray KE et al (2010) Does usnic acid affect microtubules in human cancer cells? Braz J Biol 70:659–664

Ogmundsdóttir HM, Zoëga GM, Gissurarson SR, Ingólfsdóttir K (1998) Anti-proliferative effects of lichen-derived inhibitors of 5-lipoxygenase on malignant cell-lines and mitogen-stimulated lymphocytes. J Pharm Pharmacol 50:107–115

Oksanen I (2006) Ecological and biotechnological aspects of lichens. Appl Microbiol Biotechnol 73:723–734

Pejin B, Iodice C, Bogdanović G et al (2013) Stictic acid inhibits cell growth of human colon adenocarcinoma HT-29 cells. Arab J Chem. doi:10.1016/j.arabjc.2013.03.003

Rankovic BR, Kosanic MM, Stanojkovic TP (2011) Antioxidant, antimicrobial and anticancer activity of the lichens *Cladonia furcata, Lecanora atra* and *Lecanora muralis*. BMC Complement Altern Med 11:97

Rankovic B, Kosanic M, Stanojkovic T et al (2012) Biological activities of Toninia candida and Usnea barbata together with their norstictic acid and usnic acid constituents. Int J Mol Sci 13: 14707–14722

Rankovic B, Kosanic M, Manojlovic N et al (2014) Chemical composition of *Hypogymnia physodes* lichen and biological activities of some its major metabolites. Med Chem Res 23: 408–416

Ren MR, Hur JS, Seo KI et al (2009) Anti-proliferative effects of *Lethariella zahlbruckneri* extracts in HT-29 human colon cancer cells. Food Chem 47:2157–2162

Ribeiro-Costa RM, Alves AJ, Santos NP et al (2004) In vitro and in vivo properties of usnic acid encapsulated into PLGA-microspheres. J Microencapsul 21:371–384

Richardson DHS (1991) Lichens as biological indicators—recent developments. In: Jeffrey DW, Madden B (eds) Bioindicators and environmental management. Academic, London, pp 263–272

Romagni JG, Dayan FE (2002) Structural diversity of lichen metabolites and their potential use. In: Upadhyay RK (ed) Advances in microbial toxin research and its biotechnological exploitation. Kluwer/Plenum, New York, pp 151–169

Russo A, Piovano M, Lombardo L et al (2006) Pannarin inhibits cell growth and induces cell death in human prostate carcinoma DU-145 cells. Anticancer Drugs 17:1163–1169

Russo A, Piovano M, Lombardo L et al (2008) Lichen metabolites prevent UV light and nitric oxide-mediated plasmid DNA damage and induce apoptosis in human melanoma cells. Life Sci 83:468–474

Russo A, Caggia S, Piovano M et al (2012) Effect of vicanicin and on human prostate cancer cells: role of Hsp70 protein. Chem Biol Interact 195:1–10

Sahu SC, Amankwa-Sakyi M, O'Donnell MW Jr et al (2011) Effects of usnic acid exposure on human hepatoblastoma HepG2 cells in culture. J Appl Toxicol. doi:10.1002/jat.1721

Shibamoto T, Wei CI (1984) Mutagenicity of lichen constituents. Environ Mutagen 6:757–762

Shibata S, Nishikawa Y, Tanaka M et al (1968) Antitumour activities of lichen polysaccharides. Z Krebsforsch 71:102–104

Shrestha G, St. Clair LL (2013) Lichens: a promising source of antibiotic and anticancer drugs. Phytochem Rev 12:229–244

Srinivas G, Babykutty S, Sathiadevan PP et al (2007) Molecular mechanism of emodin action: transition from laxative ingredient to an antitumor agent. Med Res Rev 27:591–608

Stocker-Wörgötter E (2008) Metabolic diversity of lichen-forming ascomycetous fungi: culturing, polyketide and shikimate metabolite production, and PKS genes. Nat Prod Rep 25:188–200

Stocker-Wörgötter E, Elix JA (2002) Secondary chemistry of cultured mycobionts: formation of a complete chemosyndrome by the lichen fungus of *Lobaria spathulata*. Lichenologist 34: 351–359

Takai M, Uehara Y, Beisler JA (1979) Usnic acid derivatives as potential antineoplastic agents. J Med Chem 22:1380–1384

Tokiwano T, Satoh H, Obara T et al (2009) A lichen substance as an antiproliferative compound against HL-60 human leukemia cells: 16-O-acetyl-leucotylic acid isolated from Myelochroa aurulenta. Biosci Biotechnol Biochem 73:2525–2527

Williams DE, Bombuwala K, Lobkovsky E et al (1998) Ambewelamides A and B, antineoplastic epidithiapiperazinediones isolated from the lichen *Usnea sp*. Tetrahedron Lett 39:9579–9582

Yamamoto Y (2000) Screening of biological activities and isolation of biological active compounds from lichens. Chem Regul Plants 35:169–179
Yamamoto Y, Miura Y, Higuchi M et al (1993) Using lichen tissue cultures in modern biology. Bryologist 96:384–393
Yamamoto Y, Kinoshita Y, Kurokawa T et al (1995) Cell growth and pigment production in suspension cultures of a mycobiont isolated from the lichen Cladonia cristatella. Can J Bot 73: 590–594
Yoshimura I, Kurokawa T, Kinoshita Y et al (1994) Lichen substances in cultured lichens. J Hattori Bot Lab 76:249–261