Pharmacologically Active Natural Compounds for Lung Cancer

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Abstract
This article consists of an analysis of the available scientific research on botanically derived compounds that have potential efficacy in the treatment of lung cancer. The mechanisms of activity reviewed include alkylating agents, topoisomerase poisons, DNA synthesis inhibitors, protein synthesis inhibitors, immunoceuticals, and lipoxygenase inhibitors. Selection criteria include: (1) products whose activity have at least minimal scientific confirmation – preclinical (*in vitro*, *in vivo*) or clinical; (2) products with a well-defined chemical composition; or (3) products with a well-known or scientifically plausible mechanism of activity.
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Introduction

A Medline search was performed to identify herbal compounds (well-defined chemical entities or plants extracts) with activity against lung cancer. Appropriate keywords and Boolean operators were utilized, such as "lung AND cancer AND plants" and "herbal AND lung AND cancer." In most cases, the full-text article was consulted; for a smaller number of cases, abstracts were used (articles less recent than 1990 or in Asian languages); and, in a few instances, secondary references were identified in other accessible articles.

Compounds for which there is no known mechanism of activity were not reviewed. In some compounds, apoptotic mechanisms were identified. However, since apoptosis is only a consequence of a primary mechanism – such as DNA alkylation, poisoning of DNA topoisomerases, or interference with tubuline metabolism – only those with identified primary mechanisms were considered for this review.

An Overview of Lung Cancer

Lung cancer is currently the most common cancer worldwide, comprising 17 percent of cancers in men (twice the worldwide rate of the second-most common cancer in men – prostate cancer) and 19 percent of cancers in men in developed countries. In women, lung cancer is the third-most common cancer worldwide, after breast and colorectal cancers (accounting for seven percent of all cancers in women).[1] Since 1950 in the United States, lung cancer has been the most common cause of cancer deaths in men, and in the 1980s it surpassed breast cancer as the most common cause of cancer death in women. As of 2004, lung cancer continues to account for the highest number of cancer deaths in the United States (32% men; 25% women), although current estimates show new lung cancer cases comprise only 13 and 12 percent of all cancers in the United States, in males and females, respectively.[2] Despite some progress in recent decades, lung cancer remains the leading cause of death from cancer in most

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developed countries, including the United States and the United Kingdom, as well as a leading cause of death in general.[3-4] The outlook for patients following diagnosis is poor – 80 percent die within one year with only 5-15 percent surviving five years.[4]

Lung cancer is usually classified as non-small cell lung cancer (NSCLC) or small cell lung carcinoma (SCLC). SCLC accounts for 20-25 percent of all bronchogenic malignancies,[5] and is characterized clinically by a propensity for early metastases and initial high response rates to chemotherapy and radiotherapy.[6] NSCLC is less sensitive to chemotherapy, with curative intent surgical resection the treatment of choice.[5] Most SCLCs acquire multi-drug resistance, whereas NSCLCs tend to be intrinsically resistant to chemotherapy.[7] Less than five percent of SCLC patients currently survive five years past initial diagnosis; whereas, the five-year survival rate for patients diagnosed with NSCLC is 15 percent.[5]

Extensive, worldwide epidemiological data has established cigarette smoking as the key cause of lung cancer.[8] In addition, occupational causes include radon in indoor environments, arsenic, asbestos, chromates, chloromethyl ethers, nickel, polycyclic aromatic hydrocarbons, and other agents. Outdoor air pollution (combustion-generated carcinogens) is another risk factor.[9]

Conventional Treatment

Conventional treatment of either form of lung cancer is fairly ineffective. Historically, for NSCLC prior to the development of cisplatin (when alkylating agents and antimetabolites were used), response rates rarely exceeded 20 percent nor were survival rates improved.[10-11] Later, during the cisplatin-combination era (cisplatin plus vinca alkaloid or cisplatin plus etoposide), response rates of 20-40 percent and better survival rates were attained, quality of life was improved, and symptoms were alleviated in most patients.[10-11] During the post-cisplatin era (after 1990), new antitumor drugs, such as gemcitabine, vinorelbine, paclitaxel, docetaxel, and CPT-11, were introduced with the hope of increasing response rates to as high as 50-60 percent.[10,11] The effectiveness of these agents is generally better than the combinations used during the cisplatin era, although the response rate has not been as high as initially hoped. In approximately 40 phase II studies, overall response rates were as high as 35-47 percent.[12] However, especially for stage III and IV cancers, the increase in lifespan can only be measured in months. D.H. Johnson accurately described these recent therapeutic advances as modest, stating that, "The overwhelming majority of NSCLC patients continue to die of their underlying malignancy, leaving considerable room for further refinement in the management of this disease."[13]

In the case of patients with SCLC, a distinction should be drawn between those with limited stage disease (LD) and those with extensive disease (ED). Limited disease is potentially curable, with the response to combined modality therapy as high as 85-95 percent and a complete remission rate of 50-60 percent. The median survival for these patients is 20 months, with a two-year survival of 40 percent.[14] The regimens most often used are: etoposide + cisplatin (EP; the standard regimen in the United States); cyclophosphamide, doxorubicin, and vincristine (CAV); cyclophosphamide, doxorubicin, and etoposide (CAE); or cyclophosphamide, doxorubicin, vincristine, and etoposide (CAVE).[15,16] Despite improvement in the treatment of LD-SCLC, there has been little progress for extensive disease,[14] where the same standard regimens as for LD are commonly administered.[15,17-18] For these patients, topoisomerase I inhibitors (topotecan and irinotecan) seem effective, as either first-line or second-line agents. However, "effective" in this context does not imply a miracle; for instance, with topotecan as a second-line agent, the response rates are 20-25 percent.[14,16]

Mechanisms of Activity of Antitumor Agents

Currently it is known that antitumor agents can arrest cell division by one or several mechanisms, the most important being:

▲ Microtubule Interference
▲ Topoisomerase Poisoning or Topoisomerase Catalytic Inhibition
▲ DNA Alkylation
▲ DNA Inhibition
▲ Protein Synthesis Inhibition
▲ Immune Mechanisms
▲ Lipoxygenase Inhibition

### *Microtubule Interfering Substances*

Microtubules are dynamic, polymeric structures, which (besides other biological functions) are major constituents of the mitotic spindle, the latter being essential for the separation of chromosomes during mitosis. Chemically, they are polymers of certain heterodimers of alpha- and beta-tubuline.[19,20] A range of secondary metabolites of plants interfere with the process of assembling and disassembling of microtubules, resulting in the arrest of cells in mitosis and apoptosis. The most significant are the Vinca alkaloids, colchicine, maytansine, and paclitaxel.[19] Both *Vinca* alkaloids (vincristine, vinblastine, and especially the second generation vinorelbine) and taxanes (paclitaxel, and especially docetaxel), largely used as antitumor agents in the conventional treatment of lung cancer, are derived by semisynthesis of natural plant compounds.

Broadly, at least two types of microtubule interferences have been described so far:

Blocking of the polymerization process by Vinca alkaloids (vincristine, vinblastine) and colchicine, which bind reversibly to beta-tubuline in the disassembled tubuline heterodimers; or

blocking of the disassembling of microtubules by taxanes (paclitaxel, docetaxel), which bind themselves reversibly to beta-tubuline in the polymerized microtubules.

Figure 1. Structure of the Microtubule Inhibitor Extract from *Xanthium strumarium*: 8-Epi-xanthatin
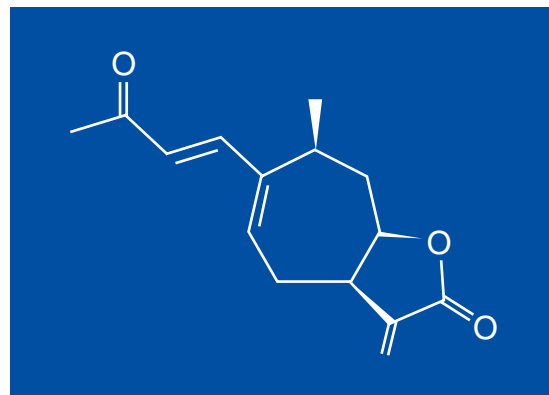
Regarding lung cancer, a methanolic extract of the leaves of *Xanthium strumarium* L. (Asteraceae) exhibited a strong inhibition of the proliferation of cultured human tumor cells, including A549 NSCLC cell line. The active constituents have been identified as 8-epi-xanthatin (Figure 1) and its epoxide, two xanthanolide sesquiterpene lactones. Their IC_{50} values have been calculated as 4.5 and 3.0 microM respectively, where the positive control cisplatin was 4.7 microM. (IC_{50} is the concentration of a compound needed to reduce growth of a population of cells by 50 percent *in vitro*. At higher concentrations (64 and 58 mM, respectively) the two xantholides showed a promising farnesyltransferase (FTase) inhibitory effect.[21] Farnesylation of certain oncoproteins (especially Ras proteins) is required for their oncogenic activity, and thus FTase inhibition could specifically stop Ras-mediated cellular proliferation. Synthetic FTase inhibitors have demonstrated activity against various human cancer cell lines, including NSCLC.[22] An earlier study showed *X. strumarium* extracts are able to effectively inhibit tubuline polymerization in mammalian tissues,[23] which could be a plausible explanation of these findings.
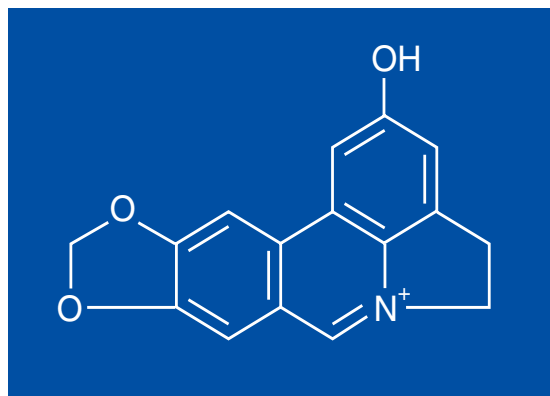
Topoisomerase Poisons

Topoisomerases are key nuclear enzymes that modify DNA topology for the efficient processing of genetic material, mainly for DNA replication and cell division. During DNA replication torsional strains are created in the double helix. Topoisomerases resolve those strains by creating transient breaks in single or both DNA strands and then rejoining the strands.[24] In their catalytic process they form a topoisomerase-DNA complex through a reversible reaction. In rapidly proliferating cells (such as cancer cells), the levels of topoisomerases are usually elevated and thus blocking their activity should hinder the proliferative process.[25]

As stated above, topoisomerases cleave one or both strands of the DNA double helix. Accordingly, topoisomerases are divided in two large classes: type I (cleave single DNA strand) and type II (cleave both DNA strands).[26] Agents that target topoisomerases (irrespective of type) are classified as topoisomerase poisons (most of the commonly used drugs) and topoisomerase catalytic inhibitors (less commonly used in therapy so far, although several substances have been developed and tested for therapeutic purposes). Topoisomerase poisons create an irreversible reaction through the creation of a drug-topoisomerase-DNA complex, thus stopping topoisomerase activity. Topoisomerase catalytic inhibitors act to impede the catalytic activity of enzymes, independently of the formation of such cleavable complexes.[27]

Many topoisomerase poisons have intercalating abilities in the DNA strands. Drugs are accordingly classified as intercalating (e.g., doxorubicine, amsacrine, ellipticine, protoberberines) or non-intercalating (e.g., etoposide, teniposide) agents.[27] Typical topoisomerase I poisons are topotecan and irinotecan, two semisynthetic derivatives of camptothecin, an alkaloid isolated from *Camptotheca acuminata* (Nyssaceae). The most important topoisomerase II poison is etoposide, a semisynthetic derivative of a natural lignan (podophyllotoxin) found in Podophyllum species (Berberidaceae).

Lycobetaine (ungeremine, AT-1840), a quaternary phenanthridinium alkaloid from *Lycoris radiata* (Amaryllidaceae), was reported to be antineoplastic. In experimental studies, intravenous injection of this alkaloid was found to inhibit (*inter alia*) Lewis lung carcinoma (LLC) in laboratory animals.[28,29] Lycobetaine (Figure 2) was cytotoxic to LXFL 529L cell line (IC_{50} = 1.2 mM[29] and intraperitoneal administration resulted in a significant growth delay of the tumors using 30 mg/kg on days 1-5 and 8-12 to nude mice bearing the large cell lung carcinoma LXFL529.[28] At growth inhibitory concentrations, lycobetaine inhibited topoisomerases I and II, stabilized the covalent DNA-topoisomerase I intermediate (the so-called cleavable complex), and induced apoptosis.[2]

Figure 2. Structure of the Topoisomerase Inhibitor Lycobetaine

In other tumor cells, lycobetaine also acted as a selective topoisomerase IIB poison.[29] Lycobetaine belongs to the class of intercalating agents, intercalating with DNA base pairs, especially the GC-pair (guanine-cytosine).[28,30,31] The betaine and a methylenedioxy group in lycobetaine are purported to be critical for its antitumor activity. The quaternary nitrogen atom in lycobetaine plays an important role in the formation of hydrogen bonds between the compound and the oligonucleotide.[24]

Another natural topoisomerase poison is nitidine, the well-known benzophenanthridine alkaloid found in the root of *Zanthoxylum nitidum* (Roxb.) DC (Rutaceae). Among others, nitidine exhibited antitumor activity against LLC in mice.[28] It is usually classified as a topoisomerase I and II poison, being a DNA intercalator;[32] however, although experimentally nitidine was able to poison topoisomerasc II, the concentrations needed are over 100 times higher than those necessary for the poisoning of topoisomerase I (40mM and 0.15 - 0.3 mM, respectively).[33] Clinical trials with nitidine were terminated for unclear reasons, although it is likely they were terminated due to unacceptable toxicity.[34]

The Annonaceous acetogenins are polyketide-derived fatty acids isolated from plants of the Annonaceae family of tropical and subtropical trees. They represent a growing class of natural compounds demonstrating a broad gamut of biological activities, including antitumor and cytotoxic effects, some of them being shown to act as topoisomerase poisons. Montanacin C, gigantetrocin A, gigantetrocin B, and annonacin, isolated from the leaves and fruits of *Annona montana,* have shown a strong cytotoxicity against LLC cells at $ED_{50} < 0.1$ mg/mL, while adriamycin, used as a positive control, had an $ED_{50} = 0.15$ mg/mL (ED_{50} represents the dose of a compound pharmacologically effective for 50 percent of the population exposed to that compound or that produces a 50-percent response in a biological system exposed to that compound.) The most active compound was annonacin, with $ED_{50} = 0.12$ mg/mL. Although these acetogenins were strongly cytotoxic against LLC cells, they were significantly less active against Meth-A cells (mouse sarcoma), and their activity was inferior to that of adriamycin.[35] Annonacin acts through apoptotic mechanisms, activating p21 in a p53-independent manner, inducing proapoptotic Bax expression and enhancing caspase 3 activity.[36] Other acetogenins like bullatacin (*Annona atemoya, A. reticulata*)[37,38] or squamocin (*A. reticulata*)[39] also activate apoptosis. The induction of apoptosis is not a distinct mechanism of activity, but rather the end result of a variety of mechanisms targeting different cell components. An experimental study suggests the primary mechanism could be that of topoisomerase poisons – annonacin (from *A. murricata, A. reticulata, Goniothalamus giganteus*) and rolliniastatin-1 (another acetogenin from *Rollinia sp.*) having such properties.[40]

Other compounds seemingly acting as topoisomerase inhibitors are baicalein (a flavonoid found in *Scutellaria baicalensis* Georgi) and ursolic acid (a triterpenic derivative). Interestingly, a catalytic inhibitor of topoisomerase IIa, demethylzeylasterone, (a triterpenoid from *Kokoona zeylanica*) was inactive against a NSCLC cell line (NCI-H460), while active on a breast cancer cell line MCF-7.[41]

DNA Alkylating Agents

The main topoisomerase poisons or microtubule-interfering agents are developed from plant compounds (most of them being semisynthetic derivatives of such compounds). Unlike the above mechanisms, alkylating agents from natural sources have not been well investigated. There has been some investigation into the natural alkylating agent 4-ipomeanol, but investigation was suspended after the first clinical trials.

4-Ipomeanol is a pneumotoxic furan derivative isolated from the sweet potato *Ipomoea batatas* (Convolvulaceae) infected with the fungus *Fusarium solani*.[42] It was the first cytotoxic agent to undergo clinical development based on a biochemical-biological rationale as an antineoplastic agent targeted specifically against lung cancer.[43] 4-Ipomeanol's metabolic activation and intracellular binding, as well as cytotoxicity, occurs selectively in normal or neoplastic tissues rich in specific P450 mixed function oxidase enzymes. Although such tissues include liver, kidney, and lung, in experimental animals 4-ipomeanol exhibited activation, covalent binding, and cytotoxicity preferentially in lung tissue (and experimentally prominent in NSCLC, but not in SCLC cells).[44] At the doses used in preclinical studies such effects were not observed in liver or kidney.[33,45] 4-Ipomeanol is activated by the cytochrome P450 system to an alkylating metabolite,

which then acts like the classic alkylating agents.[46,47] But contrary to what preclinical studies had suggested, the main toxicity was hepatocellular, not at the level of the lung.[43] Disappointingly, in clinical settings the results were very unsatisfactory with no objective antitumor responses and toxicity concerns.[43,48]
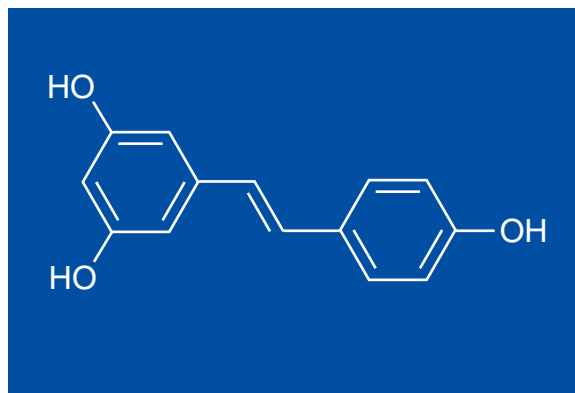
DNA Synthesis Inhibitors

　　Inhibition of DNA synthesis implies inhibition of cell multiplication, and hence, antitumor activity. However, numerous mechanisms may produce the same end result – inhibition of DNA synthesis. Thus, antimetabolite drugs (fludarabine, 5-FU, various nucleoside analogues, and hydroxyurea),[49-52] inhibitors of topoisomerase (camptothecin, topotecan),[53,54] alkylating agents (cisplatin, temozolomide, but not melphalan),[55-57] and intercalating agents (doxorubicin, daunorubicin, and others[58]) interfere with DNA synthesis. In contrast, substances interfering with tubuline (Vinca alkaloids, taxotere)[59,60] are not known to inhibit DNA synthesis, but interfere with other processes of cell division. There are also specific inhibitors of DNA polymerases, such as aphidicolin, used in experimental pharmacology and biochemistry.[61] Therefore, if a substance is found to inhibit DNA

Figure 3. Structure of Resveratrol: A DNA Synthesis and Angiogenesis Inhibitor

synthesis, the mechanism of activity is not necessarily completely understood. In the literature surveyed, several substances experimentally active in lung cancer (*in vivo* or *in vitro*) by inhibition of DNA synthesis were identified.

　　Resveratrol (Figure 3), is a polyphenol synthesized as a glycoside (resveratrol 3-O-beta-D-glucoside or piceid)[62] from certain Polygonum species (Polygonaceae). It is produced by a wide variety of plant species, including grapes, peanuts, and mulberries, and is best known for its presence in red wine. It is produced in response to injury, UV irradiation, and fungal attack, and is therefore considered a phytoalexin.[63] Resveratrol was shown to have both chemopreventive properties and antitumor activity against lung cancer at intraperitoneal doses of 2.5 and 10 mg/kg once daily (but not at 0.6 mg/kg). Resveratrol significantly reduced the tumor volume and tumor weight (42% and 44%, respectively) in mice bearing highly metastatic LLC tumors. This activity was at least partially related to the inhibition of DNA synthesis and angiogenesis through the inhibition of vascular endothelial growth factor (VEGF), a proangiogenic compound. An increase of apoptosis and decrease of S-phase population was observed for resveratrol in LLC cells, but at 10 times higher concentrations than those required for the inhibition of DNA synthesis or antiangiogenic effects.[64]

　　Indirubin (isoindigotin, indigo red) is a bisindole derivative, the red-color isomer of indigo, and a component of *Indigofera tinctoria* (Fabaceae) found in the Traditional Chinese Medicine (TCM) Qing Dai, which has been effectively used as an antileukemic agent. Indirubin is also extracted from the leaves of *Baphicacanthus cusia* (Acanthaceae), *Polygonum tinctorium* (Polygonaceae), *Isatis indigotica* (Brassicaceae), and *Indigofera suffrutticosa* (Fabaceae). While the powdered leaves contain a high level of the dye indigo blue, the antileukemic activity has been attributed to indirubin, which has also inhibited LLC in mice.[28] Indirubin exerts its anticancer effects by inhibition of DNA

polymerase I, and hence, of DNA synthesis. It inhibited DNA synthesis in several cell lines, in a cell-free assay, and *in vivo* in rats with Walker-256 sarcoma.[28,65] Indirubins are potent inhibitors of cyclin-dependent kinases (CDK), a family of key cell-cycle regulators.[66]

The alimentary uses of grapes (*Vitis vinifera* L., Vitaceae) have been known for thousands of years, being one of the most commonly consumed fruits on the planet. Grape seeds are rich in polyphenols – oligomers of flavan-3-ols known as procyanidins or proanthocyanidins.[67] Grape seed proanthocyanidins are natural antioxidants with a broad spectrum of chemoprotective properties against free radicals and oxidative stress. Tested *in vitro* on A-427 human lung cancer cells, a proanthocyanidinic extract from grape seeds (GSPE – Grape Seed Proanthocyanidin Extract) exhibited some inhibition of tumor cells (under 50 percent, depending of the concentration and time of observation). Nevertheless, GSPE had the advantage of favoring the growth and viability of normal cells.[68,69] Research on other tumor cells suggests inhibition of DNA synthesis is involved in the proanthocyanidin's antitumor activity. In human prostate carcinoma DU145 cells, a grape seed extract inhibited epidermal growth factor receptor activation by epidermal growth factor (EGF), and also exhibited DNA synthesis inhibition in starved and EGF-stimulated cells, as well as apoptosis. The results suggest the anticancer effects of the extract in this cell line are mediated via impairment of the mitogenic signals, with growth inhibition and apoptosis.[70] Investigations on breast cancer cells suggest GSPE irreversibly inhibits the growth of these cells, not as a result of apoptosis, but as a consequence of the inhibition of mitogen-activated protein kinase activation and of the induction of the cyclin-dependent kinase inhibitor Cip1/p21.[67]

Hederacolchicoside A1 (an oleanolic acid monodesmoside) isolated from *Hedera colchica* K. Koch (Araliaceae) and beta-hederin (another monodesmoside oleanolic acid, isolated from *Hedera helix* and other species) have demonstrated effective cytotoxicity against human lung cancer cell line A549, with similar IC_{50} of about 10 mM (cisplatin, the positive control tallied 5 mM). Unfortunately, it was found to affect cancerous and normal cells, as similar cytotoxicity was observed for human fibroblasts.[71] In human monocytes, hederacolchisoside A1 exerted a potent antiproliferative effect through DNA synthesis inhibition (suggesting no selectivity toward normal cells).[72] Therefore, nucleic acid inhibition could be its mechanism of activity in cancer cells.

alpha-Hederin (kalopanaxsaponin A) was identified as the active antitumor compound from a fraction of an ethanolic extract obtained from *Nigella sativa* L. seeds. Tested *in vivo* in BDF1 mice implanted with LLC, alpha-hederin exhibited a dose-dependent antitumor activity more potent than cyclophosphamide. The substance was given i.p. for seven days after tumor formation, and on day 15 its tumor inhibition rate (at a dose of 10 mg/kg body weight) was 71 percent, while the tumor inhibition rate for cyclophosphamide (20 mg/kg body weight) was only 42 percent.[73] If the observations of Barthomeuf et al are consistent, we should expect beta-hederin to be even more active that alpha-hederin (*in vitro* Barthomeuf et al observed beta-hederin was more active than alpha-hederin against the A549 cell line).[71]

In another study, alpha-hederin *in vitro* was 10 times more potent than cisplatin against 3LL Lewis lung carcinoma (ED_{50} of alpha-hederin – 1.1 mM, and ED_{50} of cisplatin – 11.3 mM). However, *in vivo*, on LLC-bearing mice, cisplatin was more active at 3 mg/kg than alpha-hederin administered i.p. at 15 mg/kg, although the difference between the effects of the two compounds was not statistically significant. It is possible that *in vivo* a significant amount of the alpha-hederin was biotransformed and inactivated. It is reasonable to conclude on the basis of this experiment, however, that alpha-hederin at 15 mg/kg has antitumor activity on 3LL Lewis lung carcinoma-bearing mice comparable to cisplatin at 3 mg/kg.[74]

alpha-Hederin may act through induction of apoptosis, since in murine leukemia P388 cells it caused a dose- and time-dependent increase in apoptosis.[75] In another experiment, alpha-hederin increased nitric oxide (NO) secretion in mouse

Figure 4.　Structure of Ursolic Acid
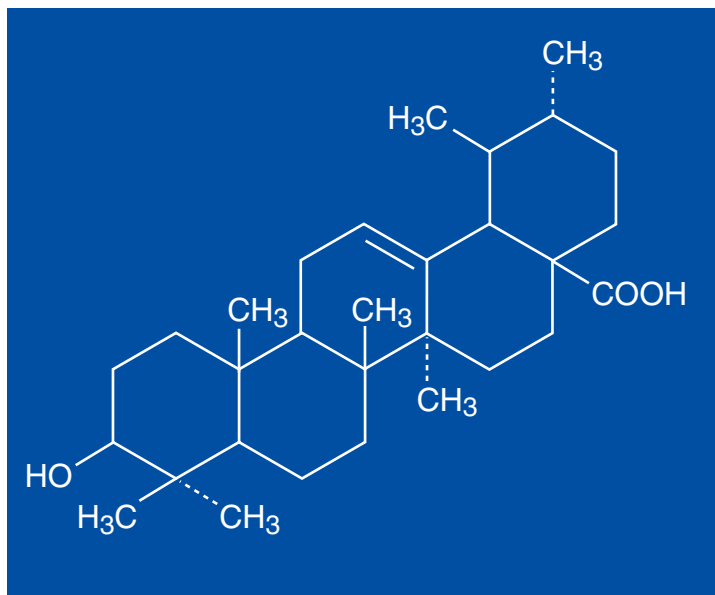
macrophages,[76] NO being a pro-apoptotic factor. This observation is also consistent with a possible induction of apoptosis by these saponins. But more specifically, it probably acts through inhibition of DNA synthesis, since alpha-hederin (similar to hederacolchicoside A1) inhibits proliferation of human monocytes via this mechanism.[72] In addition, it was experimentally shown that alpha-hederin inhibits DNA, RNA, and protein synthesis in P388 cells in a dose- and time-dependent manner.[75]

Of seven triterpenes isolated from the stem bark of *Physocarpus intermedius* Schn. (Rosaceae), 3-O-caffeoyloleanolic acid, betulinic acid, and the methyl ester of euscaphic acid (in order of decreasing potency) were the most active *in vitro* against A549 lung cancer cell line (ED_{50} values of 1.6, 2.0, and 3.7 mg/mL; ED_{50} for cisplatin 11.4 mg/mL). Since ursolic acid was found in a relatively higher concentration in the extract, the authors hypothesized this compound is responsible for the strong cytotoxicity of the extract (ED_{50} = 4.2 mg/mL against A549 cell line).[77]

Ursolic acid (Figure 4) is an inhibitor of DNA synthesis, while betulinic acid is known only to trigger apoptosis in several cell lines (but its primary mechanism is unknown). Ursolic acid was found to be a potent inhibitor of calf DNA polymerase alpha, rat DNA polymerase beta, and human DNA topoisomerases I and II, but seemingly not as a topoisomerase poison, but as a catalytical inhibitor.[78] One or all of these mechanisms combined trigger the apoptotic machinery, since ursolic acid is known to induce apoptosis in several cancer cell lines (HL-60 human promyelocytic leukemia cells,[79-81] lymphoma Daudi cells,[82] SNU-1 human stomach malignant cells,[77] A431 human epidermoid carcinoma,[83] and HepG2 human hepatoblastoma cells[84]). There may be involvement of apoptotic mechanisms in its activity against A549 cell lines. Ursolic acid isolated from the bark and stem of *Polylepis racemosa* R&P (Rosaceae) exhibited no significant cytotoxicity on the H460 cell line (large cell lung carcinoma), although pomolic acid, another triterpene isolated as the main active antitumor compound from the same plant, had a GI_{50} of 15 mg/mL on H460 tumor cells (GI_{50} refers to cell growth inhibition by 50 percent compared to untreated controls). In terms of National Cancer Institute recommendations, this means virtually no significant cytotoxicity.[85]

Apoptosis induced by ursolic acid is associated with enhanced intracellular Ca^{2+} signals[78,81,82] and proteolytic activation of caspase-3 and/or other similar caspases.[83] In human prostate epithelial cells, apoptosis is p53-independent and does not involve the change of balance between Bcl-2 and Bax expression. Unlike betulinic acid, ursolic acid seems to induce apoptosis without mitochondrial dysfunction.[86] Instead it was shown in HepG2 cells that ursolic acid increases p21(WAF1) expression, which induces the release of cytochrome c and the activation of caspase-3.[84]

Oldenlandia diffusa (Rubiaceae) is another plant experimentally active against lung cancer, from which ursolic acid was isolated as the major active compound. It is a plant used in TCM for treating liver, lung, and rectal tumors. Ursolic acid isolated from this plant exhibits significant inhibition of the proliferation of cultured tumor cells, being active against the A549 lung cancer

cell line.[87] An extract of *Oldenlandia diffusa* was cytotoxic through apoptotic mechanisms on H69 (drug sensitive) and H69VP (multi-drug resistant) lung cancer cells, and less cytotoxic on normal lung epithelial cells BEAS-2.[88] As shown above, it is likely ursolic acid triggers apoptosis through inhibition of DNA synthesis.[78,84]

In addition to this mechanism, several studies suggest *Oldenlandia diffusa* extracts are able to enhance various immune links, stimulating the immune system to kill or engulf tumor cells.[89] These extracts activated cells from lymphoid tissue to enhanced cytotoxic T-lymphocyte activity,[90,91] B-cells to produce antibodies,[89,90] and monocytes to stimulate cytokine production and phagocytosis to remove the tumor cells.[89,90] Moreover, *Oldenlandia diffusa* extracts synergistically induce pro-apoptotic NO and tumor necrosis factor-alpha (TNF-alpha) production by peritoneal macrophages when the cells are treated with recombinant interferon-gamma (rIFN-gamma).[92]

Brazilian plants also have been tested for DNA synthesis inhibition. Oncocalyxones A and C, both 1,4-anthracenediones from *Auxemma oncocalyx* (Boraginaceae), showed a weak (virtually biologically nonsignificant) cytotoxicity against SW1573 lung cell line (IC$_{50}$ = 7.0 and 7.5 mg/mL, respectively). The diterpene lactone from *Egletes viscosa* (Asteraceae), 12-acetoxy-hawtriwaic acid, was also rather weakly active against the same cancer cell line (IC$_{50}$ = 5.8 mg/mL). All three compounds caused substantial DNA damage and DNA synthesis inhibition.[93]

Several cannabinoids from marijuana have been shown to inhibit tumor growth and increase the life span of LLC-bearing mice through inhibition of DNA synthesis.[94] But given the common prohibition of Cannabis use in most countries, this information may not be of practical relevance.

Protein Synthesis Inhibitors

While many antibiotics (such as aminoglycosides, tetracyclines, chloramphenicol, macrolides, and lincosamides) are known for protein synthesis inhibitory properties, there is no important antitumor agent acting through such a

Figure 5. Structure of Methyl Rocaglate, a Novel Protein Synthesis Inhibitor
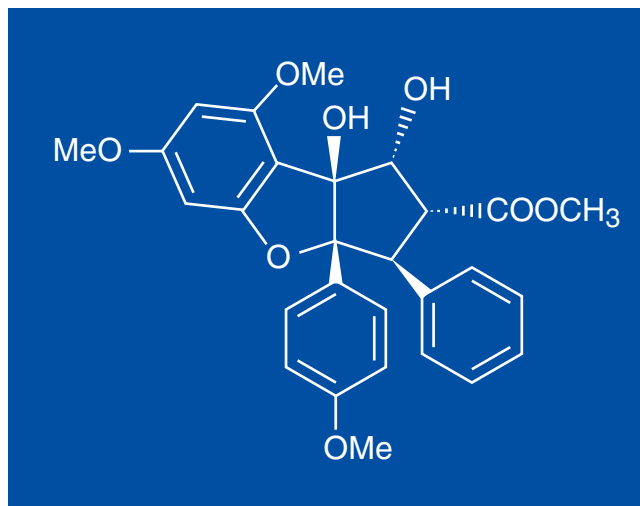
mechanism. Although a significant number of protein synthesis inhibitors are experimentally known, they are not commonly used in cancer therapy. However, a few scientific papers describe the antitumor activity of substances that act primarily through the inhibition of protein synthesis: rocaglamide-type lignans, mistletoe lectins, some alkaloids (pretazettine, lycorine, homoharringtonine, antofine, tubulosine), and some quassinoids (bruceantin).

Rocaglamide-type Lignans

Rocaglamide-type lignans (1H-cyclopenta[b]benzofuran lignans) have been isolated from more than ten species of the genus Aglaia (Meliaceae) and are exclusively confined to members of this genus.[95] Aglaia comprises 130 species of dioecious trees or shrubs distributed mainly in tropical and subtropical regions.[96] Recently, Aglaia species have attracted scientific interest due to their unique rocaglamide lignans. Several rocaglamide-type lignans, isolated from the stems of *Aglaia elliptica* B1, exhibited cytostatic (not cytotoxic) properties against several cancer cell lines, including Lu1 (human lung carcinoma). In particular, 4'-demethoxy-3',4'-methylenedioxy-methyl rocaglate was subject to additional investigations that revealed the

compound inhibits protein synthesis, but not nucleic acid biosynthesis. Administered in mice at 10 mg/kg body weight, no signs of overt toxicity were observed.[97,98] Four such cyclopenta[*b*] benzofuran lignans isolated from the stem bark of the Formosan plant *Aglaia formosana* (Hayata) exhibited significant cytotoxic activity against an A549 lung cancer cell line, as well as against several other cancer cell lines. Methyl rocaglate (Figure 5) was the most active, with an ED_{50} of 0.0094 mg/mL (ED_{50} for mithramycin used as a positive control was 0.07 mg/mL). The other three compounds were also more potent than the control substance, having ED_{50} less than mithramycin.[99] Independent researchers have confirmed on several cancer cell lines the cytostatic and protein synthesis inhibitory activities of rocaglamide lignans.[100,101]

Mistletoe Lectins

Extracts from mistletoe (*Viscum album*, the well-known parasitic plant belonging to the Loranthaceae family) have been used against a variety of diseases, but several extracts marketed under names such as Iscador[102] and Isorel[103] are widely used as complementary cancer remedies in Europe. Although there are still doubts concerning efficacy in curing or improving cancer, significant research has been dedicated to these extracts. Mistletoe contains three type-2 ribosome-inactivating proteins known as mistletoe lectins (ML1, ML2, ML3).[104,105] The best characterized of these is ML1, also known as viscumin or *V. album* agglutinin-I (VAA-I), with similar structure and mechanism of action as abrin, ricin, and modeccin.[102,106,107] Viscumin consists of two A-chains and two B-chains linked by a disulfide bond and binds specific to galactoside structures. ML2 can bind to either N-acetyl-D-galactosamine or D-galactose; whereas, ML3 is specific for N-acetyl-galactosamine.[108] The lectins are often identified as the main active principals of mistletoe without distinguishing among the three.

Regarding the use of mistletoe in lung cancer, several published articles focus on antimetastatic properties of mistletoe extracts. In a single paper, ML1 enhanced the cytotoxic effects of several chemotherapeutic drugs, including doxorubicin, cisplatin, and taxol, in human lung carcinoma cell line A549. The combination of ML1 and cycloheximide (a known protein synthesis inhibitor) showed strong synergistic effects.[109] In another experiment, the mistletoe extract marketed as Isorel inhibited protein synthesis in various malignant cell lines, suggesting this could be one possible mechanism of mistletoe extracts' activity. According to *in vitro* results, inhibition of protein synthesis could be the result, not only of the lectins and other high molecular weight factors, but also of some substances with very low molecular weight (< 500 Da).[103]

Alkaloids

Pretazettine, an alkaloid unique to species (such as Narcissus) belonging to the family Amaryllidaceae, has been found active against LLC (as well as other forms of cancer), and it has also been reported to inhibit protein synthesis in eukaryotic cells.[110]

Lycorine, an Amaryllidaceous phenanthridine alkaloid related to lycobetaine, has been found cytotoxic against the large cell lung carcinoma LXFL529[28] and against LLC (for the latter $ED_{50} = 0.5$ mg/mL).[111] It was tested *in vivo* in BDF-1 mice inoculated subcutaneously with LLC cells. When the mice were treated for two weeks, lycorine at 10 mg/kg exhibited antitumor activity, with a tumor inhibition rate of 80.5 percent on day 19. (Tumor inhibition rate calculated by dividing the mean tumor volume of the treatment group by the mean tumor volume of the control group, and multiplying the result by 100.) The alkaloid decreased the body weight of the treated animals by five percent compared to control. Body weight gradually increased, however, during the course of treatment to the range of the control at the end of administration.[111] Lycorine, although structurally and biogenetically related to lycobetaine, inhibited protein synthesis[112] but did not exhibit DNA intercalating abilities.[28] Lycorine is known to inhibit protein synthesis in eukaryotic cells, as well as in cell-free systems in which protein synthesis is mediated

by eukaryotic ribosomes.[113] Experiments conducted on mouse lymphoma cells suggest lycorine could exert its antiproliferative effects through complex formation with tRNA.[110]

Homoharringtonine is an ester of cephalotaxine, both alkaloids derived from *Cephalotaxus harringtonia* (Cephalotaxaceae) a Chinese coniferous tree. Human colon and lung adenocarcinoma cell lines resistant to multi-drug therapy are sensitive to homoharringtonine treatment. Homoharringtonine inhibits synthesis of both proteins and DNA.[114,115] It inhibits protein synthesis through suppression of the elongation phase of translation by preventing substrate binding to the acceptor site on the 60-S ribosome and blocking aminoacyl-tRNA binding and peptide bond formation. In addition, it inhibits glycosylation of glycoproteins and lipid-linked oligosaccharide formation. It also experimentally suppressed the incorporation of [3H]thymidine into DNA, but it was hypothesized this DNA synthesis inhibition might be a secondary phenomenon of protein synthesis inhibition.[116] However, Efferth suggests focusing on protein biosynthesis alone may be too narrow to unravel other mechanisms of homoharringtonine's inhibitory action unrelated to protein biosynthesis.[116]

Antofine is a phenanthroindolizidine alkaloid isolated from the root of *Cynanchum paniculatum* Kitagawa (Asclepiadaceae), a small, perennial herbal plant in Asian countries. The compound exhibited a strong cytotoxicity against A549 lung cells, with an $IC_{50} < 10$ ng/mL (while the IC_{50} of ellipticine, an experimental positive control, was 500 ng/mL). As judged by flow cytometric analysis, cells were remarkably blocked in the G2/M phases of the cycle. Since some antofine analogs, tylophorine and tylocrebrine from *Tylophora crebiflora* (Asclepiadaceae), showed inhibition of protein synthesis in cancer cells, the same mechanism could be true also for antofine, but thus far this is just a conjecture.[117]

(-)-Tubulosine, an alkaloid isolated from *Pogonopus speciosus* (Jack). K. Schum. (Rubiaceae), was "extremely potent" in a cell culture panel, with the best activity demonstrated against the Lu1 human lung cancer cell line ($ED_{50} < 0.001$ mg/mL).[98] Tubulosine has been shown to interfere with protein synthesis, inhibiting the process of peptide chain elongation by specifically preventing the elongation-factor-2-dependent step of translocation, a possible mechanism of its cytotoxic activity.[118]

Quassinoids

Bruceantin, a quassinoid isolated from *Brucea antidysenteria* (Simaroubaceae), is a potent anticancer compound that decreased the growth of LLC cells. Its primary mechanism of action is the inhibition of protein synthesis.[97,114]

Compounds Acting via Immune Modulation

Several natural polymeric compounds have been isolated from various plants and from more than 50 species of mushrooms, compounds relatively well known presently for their immuno-stimulating properties. Compounds isolated from more than 30 mushroom species have shown anticancer activity in animals. Among these, PSK (Polysaccharide Kureha; Polysaccharide K; krestin) and PSP (polysaccharide P), two proteoglycans from *Coriolus versicolor,* and *Grifola frondosa* (maitake mushroom D fraction) have been shown to be effective in lung cancer treatment in clinical studies. These compounds stimulate host immunity via several pathways.[119] It has been said that "as many potential mechanisms have been identified as the many pathways known to exist in the immune system."[119] For instance, PSP is not active *in vitro* against tumor cell lines and mouse peritoneal macrophages; instead, it activates *in vivo* the transcription of TNF gene in these cells, indicating that PSP exerts an immunomodulatory effect on the defensive cells. In addition, PSP, as a biological response modifier, induced the production of IFN-alpha, IFN-gamma, and interleukin-2 (IL-2), and enhanced T-cell proliferation. It counteracted the depressive effect of cyclophosphamide on leukocyte count and showed a restorative effect against spleen injury in mice induced by gamma-irradiation.[120] This

shows that immunoceuticals such as PSP have antitumor activity and offer some protection against the side effects of chemotherapy.

In a clinical setting, lung cancer patients with stage III disease who received PSK had a better prognosis than stage I and II patients who did not receive PSK. Survival time in the PSK-administered group was significantly longer than in the non-administered group. PSK given in addition to radiotherapy was particularly helpful for older patients (>70 years) and patients with smaller primary tumors (5 cm diameter). Two-year survival rates of patients older than 70 years were 55 percent for patients who received PSK and 22 percent for patients who did not receive PSK. After five years, survival rates were 23 percent and 7 percent, respectively.[121]

Kodama et al published an informal summary of an open-label, non-randomized study on maitake MD-fraction conducted in Japan. The study was performed on 36 patients with various cancers, nine with lung cancer who had discontinued chemotherapy due to side effects. The treatment consisted of maitake D-fraction (35-100 mg daily) plus tablets of dried crude extract of maitake (4-6 grams). Symptomatic improvements or regression were claimed for five of nine lung cancer cases.[122] In a previous study Nanba also found that 83 percent of patients experienced lessening of pain and 90 percent experienced improvement

of chemotherapy-related symptoms such as vomiting, nausea, reduced appetite, hair loss, intestinal bleeding, and lowered white blood cell count.[119]

Lipoxygenase Inhibitors

It is commonly accepted that release of arachidonic acid from membrane phospholipids plays an important role in cancer cell proliferation; hence, inhibition of lipoxygenases, enzymes defining a pathway for arachidonate metabolism, could contribute to the prevention and arrest of tumor development.[123]

Baicalein, wogonin, their glucopyranosiduronides baicalin and wogonoside, and skullcapflavone II (neobaicalein), flavonoids isolated from the root of *Scutellaria baicalensis* Georgi (Lamiaceae), inhibited the growth of the human tumor cell lines LXFL 529L (large cell lung carcinoma) at a micromolar range.[28] In numerous forms of cancer, baicalein (Figure 6) acts through the inhibition of 12-lipoxygenase. Several studies demonstrated baicalein induced apoptosis in gastric,[123] pancreatic,[124] human breast,[125] and prostate[126] cancer cells through inhibition of 12-lipoxygenase. The same mechanism may apply to lung cancer.

On other tumor cells (e.g., human hepatocellular carcinoma), baicalein exhibited a strong inhibition of topoisomerase II,[127,128] although it appears the compound is also able to inhibit topoisomerase I.[28] Besides the mentioned activities, baicalein exhibits antiangiogenic activity through inhibition of matrix metalloproteinases (MMPs).[129]

Wogonin has not yet been shown to be a topoisomerase poison or 12-lipoxygenase inhibitor. It is mentioned here, however, because of its close structural and botanical connection with baicalein. Instead, it has been shown to exert its cytotoxic effects in other cancer cells through apoptosis triggering, with activation of caspase 3 and induction of p53 and p21 (Waf/Cip-1) proteins.[130,131]

Figure 6. Structure of the Lipoxygenase Inhibitor Baicalein
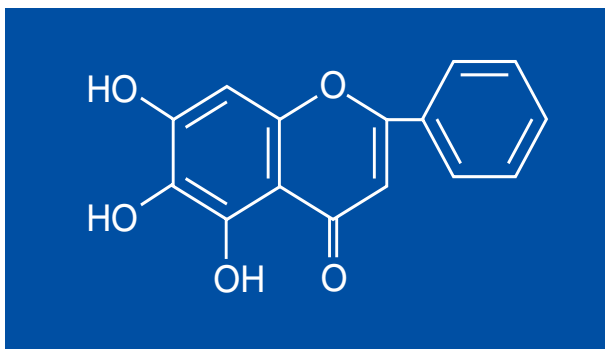
Conclusion

This review indicates a significant number of herbal compounds are potentially useful in the treatment of lung cancer. For most substances discussed in this article, the results appear promising, but they need confirmation in clinical settings since most of the experiments published thus far have been pre-clinical. The transition from pre-clinical to clinical studies is not an easy one, taking into account that usually herbal product manufacturers are small companies with limited research resources and expertise.[132] Although very promising in the pre-clinical phase, a substance could prove disappointing when administered to patients in a clinical setting, 4-ipomoeanol being such an example. Developed with great expectations for the targeted treatment of lung cancer, with good results in animal experiments, it had very poor results in clinical settings, with no objective antitumor responses and unexpected side effects. This emphasizes again the necessity of rigorous, randomized clinical trials.

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