

PLANTS AS A SOURCE OF ANTI-CANCER AGENTS

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Summary

Plant-derived compounds have played an important role in the development of several clinically useful anti-cancer agents. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin, and paclitaxel (taxol[®]). Several promising new agents are in clinical development based on selective activity against cancer-related molecular targets, including flavopiridol and combretastin A4 phosphate, and some agents which failed in earlier clinical studies are stimulating renewed interest.

1. Introduction

Plants have a long history of use in the treatment of cancer. Hartwell, in his review of plants used against cancer, lists more than 3000 plant species that have reportedly been used in the treatment of cancer. In many instances, however, the “cancer” is undefined, or reference is made to conditions such as “hard swellings”, abscesses, calluses, corns, warts, polyps, or tumors, to name a few. These symptoms would generally apply to skin, “tangible”, or visible conditions, and may indeed sometimes correspond to a cancerous condition. Many of the claims for efficacy in the treatment of cancer, however, should be viewed with some skepticism because cancer, as a specific disease entity, is likely to be poorly defined in terms of folklore and traditional medicine. This is in contrast to other plant-based therapies used in traditional medicine for the treatment of afflictions such as malaria and pain, which are more easily defined, and where the diseases are often prevalent in the regions where traditional medicine systems are extensively used. However, despite these observations, it is significant that over

60% of currently used anti-cancer agents are derived in one way or another from natural sources, including plants, marine organisms and micro-organisms. Indeed, molecules derived from natural sources (so-called natural products), including plants, marine organisms and micro-organisms, have played, and continue to play, a dominant role in the discovery of leads for the development of conventional drugs for the treatment of most human diseases.

While in past years, cancer has been regarded mainly as a group of diseases afflicting the more developed countries, the incidence of various forms of cancer is now rapidly rising worldwide. Reference to the World Health Organization database on cancer incidence and mortality [<http://www.who.int/cancer/resources/incidences/en/>] indicates substantial numbers of cases of major cancers in less developed countries (see Table 1).

Cancer Type	Number of cases in the year 2000*		
	Total	More developed countries	Less developed countries
All (except skin)	5,317,905	2,503,772	2,814,132
Breast	1,050,346	579,285	471,063
Colon/Rectum	498,574	318,694	180,059
Kidney	118,255	79,090	39,158
Leukemia	144,321	58,416	85,912
Liver	398,364	73,270	325,108
Lung	901,746	470,836	430,919
Melanoma	65,177	50,608	14,571
Oral Cavity	169,524	59,959	109,553
Ovary	192,379	91,307	101,060
Prostate	542,990	415,568	127,419
Stomach	558,458	208,282	350,176

* Numbers apply to all ages and males only, except for breast and ovary. The total numbers often do not correspond to the sums of the more and less developed countries

Table 1. The number of cases in more developed/less developed countries as of the year 2000

The search for anti-cancer agents from plant sources started in earnest in the 1950s with the discovery and development of the vinca alkaloids, vinblastine and vincristine, and the isolation of the cytotoxic podophyllotoxins (see Section 2). These discoveries prompted the United States National Cancer Institute (NCI) to initiate an extensive plant collection program in 1960, focused mainly in temperate regions. This led to the discovery of many novel chemotypes showing a range of cytotoxic activities, including the taxanes and camptothecins, but their development into clinically active agents spanned a period of some 30 years, from the early 1960s to the 1990s. This plant collection program was terminated in 1982, but with the development of new screening technologies, the NCI revived the collections of plants and other organisms in 1986. This time the focus was on the tropical and sub-tropical regions of the world, but it is interesting to note that no new plant-derived clinical anti-cancer agents have, as yet,

reached the stage of general use. However, as described in Sections 3 to 5, a number of agents are in preclinical development.

2. Plant-Derived Anti-Cancer Agents in Clinical Use (see Figure 1)

The first agents to advance into clinical use were the so-called vinca alkaloids, vinblastine (VLB) and vincristine (VCR), isolated from the Madagascar periwinkle, *Catharanthus roseus* G. Don. (Apocynaceae), which was used by various cultures for the treatment of diabetes. These drugs were first discovered during an investigation of the plant as a source of potential oral hypoglycemic agents. While research investigators could not confirm this activity, it was noted that extracts reduced white blood cell counts and caused bone marrow depression in rats, and subsequently it was found that the treatment of mice bearing a transplantable lymphocytic leukemia caused significant life extension. This led to the isolation of VLB and VCR as the active agents, so their discovery may be indirectly attributed to the observation of an unrelated medicinal use of the source plant. It is interesting to note that though the plant was originally endemic to Madagascar, the samples used in the discovery of VLB and VCR were collected in Jamaica and the Philippines. More recent semi-synthetic analogues of these agents are vinorelbine (VRLB) and vindesine (VDS). These agents are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers. VLB is used for the treatment of leukemias, lymphomas, advanced testicular cancer, breast and lung cancers, and Kaposi's sarcoma, and VCR, in addition to the treatment of lymphomas, also shows efficacy against leukemias, particularly acute lymphocytic leukemia in childhood. VRLB has shown activity against non-small-cell lung cancer and advanced breast cancer. Of over 2069 anti-cancer clinical trials recorded by the NCI as being in progress as of July 2004, over 160 are drug combinations including these agents against a range of cancers.

The two clinically-active agents, etoposide (VM 26) and teniposide (VP 16-213), which are semi-synthetic derivatives of the natural product, epipodophyllotoxin (an isomer of podophyllotoxin), may be considered as being more closely linked to a plant originally used for the treatment of "cancer". The *Podophyllum* species (Podophyllaceae), *P. peltatum* Linnaeus (commonly known as the American mandrake or Mayapple), and *P. emodii* Wallich from the Indian subcontinent, have a long history of medicinal use, including the treatment of skin cancers and warts. *P. peltatum* was used by the Penobscot Native Americans of Maine for the treatment of "cancer", and interest was promoted by the observation in the 1940s that venereal warts could be cured by topical application of an alcohol extract of the dried roots (called podophyllin). The major active constituent, podophyllotoxin, was first isolated in 1880, but its correct structure was only reported in the 1950s. Many closely related podophyllotoxin-like lignans were isolated during this period, and several of them were introduced into clinical trials, only to be dropped due to lack of efficacy and unacceptable toxicity. Extensive research at Sandoz Laboratories in Switzerland in the 1960s and 1970s led to the development of etoposide and teniposide as clinically effective agents which are used in the treatment of lymphomas and bronchial and testicular cancers. Of 2069 anti-cancer clinical trials recorded by the NCI as being in progress as of July 2004, over 150 are drug combinations including etoposide against a range of cancers.

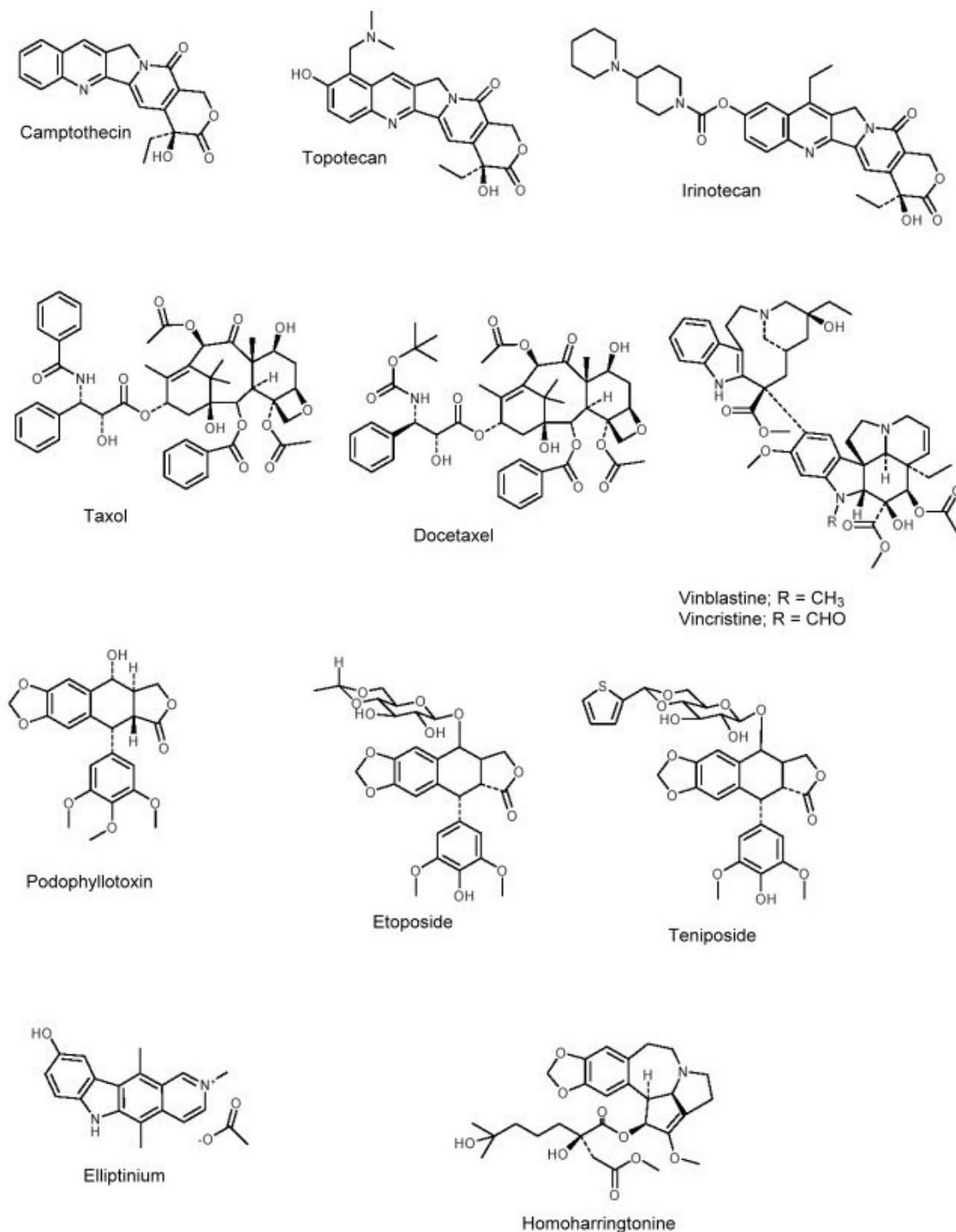


Figure 1. Plant-derived anti-cancer agents in clinical use.

A more recent addition to the armamentarium of plant-derived chemotherapeutic agents is the class of molecules called taxanes. Paclitaxel (taxol[®]) initially was isolated from the bark of *Taxus brevifolia* Nutt. (Taxaceae), collected in Washington State as part of a random collection program by the U.S. Department of Agriculture (USDA) for the National Cancer Institute (NCI). The use of various parts of *T. brevifolia* and other *Taxus* species (e.g. *T. canadensis* Marshall, *T. baccata* L.) by several Native American

tribes for the treatment of some non-cancerous conditions has been reported, while the leaves of *T. baccata* are used in the traditional Asiatic Indian (Ayurvedic) medicine system, with one reported use in the treatment of “cancer”. Paclitaxel, along with several key precursors (the baccatins), occurs in the leaves of various *Taxus* species, and the ready semi-synthetic conversion of the relatively abundant baccatins to paclitaxel, as well as active paclitaxel analogs, such as docetaxel (Taxotere[®]), has provided a major, renewable natural source of this important class of drugs. Paclitaxel is used in the treatment of breast, ovarian and non-small-cell lung cancer (NSCLC), and has also shown efficacy against Kaposi sarcoma. Paclitaxel has also attracted attention in the potential treatment of multiple sclerosis, psoriasis and rheumatoid arthritis. Docetaxel is primarily used in the treatment of breast cancer and NSCLC. The importance of this class of anti-cancer agents may be judged from the fact that 12 and 23 taxane analogs are in clinical and preclinical development, respectively. In addition, of 2069 cancer clinical trials recorded by the NCI as being in progress as of July 2004, 248 or close to 12% are listed as involving taxane-derived drugs, including 134 with paclitaxel (Taxol[®]), 105 with docetaxel (Taxotere[®]), and 10 with miscellaneous taxanes, either as single agents or in combination with other anti-cancer agents. In addition, 23 taxanes are in preclinical development.

Another important addition to the anti-cancer drug armamentarium is the class of clinically-active agents derived from camptothecin, which is isolated from the Chinese ornamental tree, *Camptotheca acuminata* Decne (Nyssaceae), known in China as the tree of joy. Camptothecin was discovered from extracts of plants originally collected by the U. S. Department of Agriculture as a possible source of steroidal precursors for the production of cortisone. The extract of *C. acuminata* was the only one of 1000 of these plant extracts tested for anti-tumor activity which showed efficacy, and camptothecin was isolated as the active constituent. Camptothecin (as its sodium salt) was advanced to clinical trials by the NCI in the 1970s, but was dropped because of severe bladder toxicity. However, extensive research was performed by several pharmaceutical companies in a search for more effective camptothecin derivatives, and Topotecan (Hycamtin[®]), developed by SmithKline Beecham (now Glaxo SmithKline), and Irinotecan (CPT-11; Camptosar[®]), originally developed by the Japanese company, Yakult Honsha, are now in clinical use. Topotecan is used for the treatment of ovarian and small-cell lung cancers, while Irinotecan is used for the treatment of colorectal cancers. Of the 2069 cancer clinical trials recorded by the NCI as being in progress, as of July 2004, 94 or approximately 4.5% are listed as involving camptothecin-derived drugs, including 64 with irinotecan (CPT-11), 26 with topotecan, and 4 with other miscellaneous analogues, either as single agents or in combination with other anti-cancer agents. In addition, 15 other camptothecin derivatives are in preclinical development.

Other plant-derived agents in clinical use are homoharringtonine, isolated from the Chinese tree, *Cephalotaxus harringtonia* var. *drupacea* (Sieb and Zucc.) (Cephalotaxaceae), and elliptinium, a derivative of ellipticine, isolated from species of several genera of the Apocynaceae family, including *Bleekeria vitensis* A. C. Sm., a Fijian medicinal plant with reputed anti-cancer properties. A racemic mixture of harringtonine and homoharringtonine (HHT) has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia.

Purified HHT has shown efficacy against various leukemias, including some resistant to standard treatment, and has been reported to produce complete hematologic remission (CHR) in patients with late chronic phase chronic myelogenous leukemia (CML). Elliptinium is marketed in France for the treatment of breast cancer.

3. Plant-Derived Anticancer Agents in Clinical Development (Figure 2)

The flavone, flavopiridol is totally synthetic, but the basis for its novel structure is a natural product, rohitukine, isolated by chemists at Hoechst India Ltd. in the early 1990s from *Dysoxylum binectariferum* Hook. f. (Meliaceae), which is phylogenetically related to the Ayurvedic plant, *D. malabaricum* Bedd., used for rheumatoid arthritis. Rohitukine was isolated as the constituent responsible for anti-inflammatory and immunomodulatory activity. A total synthesis was undertaken, and one of the over 100 analogues synthesized during structure-activity studies was flavopiridol, which was found to possess tyrosine kinase activity and potent growth inhibitory activity against a series of breast and lung carcinoma cell lines. It also showed broad spectrum *in vivo* activity against human tumor xenografts in mice, and this led to its selection for preclinical and clinical studies by the NCI in collaboration with Hoechst. It is currently in 18 Phase I and Phase II clinical trials, either alone or in combination with other anti-cancer agents, against a broad range of tumors, including leukemias, lymphomas and solid tumors. While flavopiridol alone is probably not a viable treatment, use of the compound in conjunction with other agents such as paclitaxel and cisplatin has led to partial and complete remissions in a number of Phase I patients, leading to Phase II studies in patients with a variety of paclitaxel-resistant tumors.

The combretastatins were isolated from the South African “bush willow”, *Combretum caffrum* (Eckl. & Zeyh.) Kuntze (Combretaceae), collected in Southern Africa in the 1970s for the NCI by the USDA, working in collaboration with the Botanical Research Institute of South Africa. These collections were part of a random collection program aimed at the discovery of novel anti-cancer agents. Species of the *Combretum* and *Terminalia* genera, both of which belong to the Combretaceae family, are used in African and Indian traditional medicine for the treatment of a variety of diseases, including hepatitis and malaria. Several *Terminalia* species have reportedly been used in the treatment of “cancer”. The combretastatins are a family of stilbenes which act as anti-angiogenic agents, causing vascular shutdown in tumors and resulting in tumor necrosis. A water-soluble analogue, combretastatin A-4 phosphate (CA4), has shown promise in early clinical trials. Of interest is the number of combretastatin (CA4) mimics being developed. Three are in clinical trials, while 11 are in preclinical development. This chemical class has served as a model for the synthesis of a host of analogues containing the essential trimethoxy aryl moiety (see Figure 2) linked to substituted aromatic moieties through a variety of two or three atom bridges including heterocyclic rings and sulfonamides. This is an impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry.

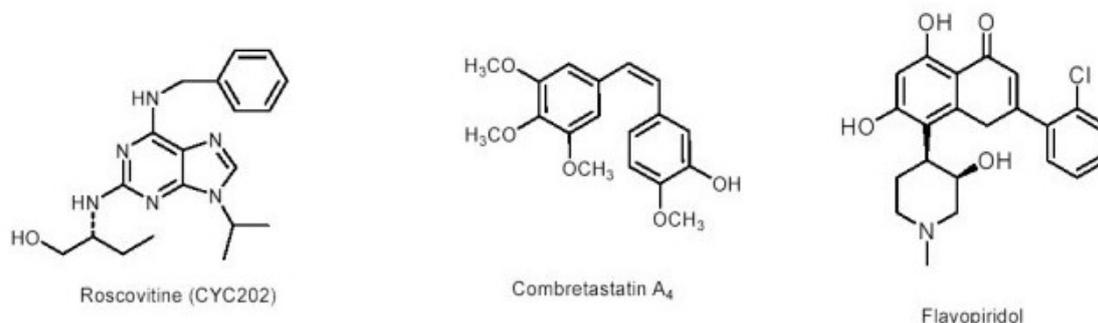


Figure 2. Plant-derived anti-cancer agents in clinical development

An interesting agent in early clinical development is roscovitine which is derived from olomucine, originally isolated from the cotyledons of the radish, *Raphanus sativus* L. (Brassicaceae), but which is now produced synthetically. Olomucine stimulated interest as a result of its inhibition of cyclin-dependent kinases (Cdk), proteins which play a major role in cell cycle progression. Chemical modification of olomucine resulted in the more potent inhibitor, roscovitine, which is in clinical development under the code CYC202 by Cyclacel in Dundee, Scotland, and currently is in Phase II clinical trials in Europe. Further development of this series, following synthesis of a focused library via combinatorial chemistry techniques, has led to the purvalanols which were even more potent, and are in preclinical development.

4. Targeting Natural Products

A recurring liability of natural products, at least in the area of cancer chemotherapy, is that, although many are generally very potent, they have limited solubility in aqueous solvents and exhibit narrow therapeutic indices. These factors have resulted in the demise of a number of pure natural products, such as the plant-derived agents, bruceantin and maytansine, as promising leads. An alternative approach to utilizing such agents is to investigate their potential as ‘warheads’ attached to monoclonal antibodies specifically targeted to epitopes on tumors of interest.

A promising case is that of maytansine. Maytansine (Figure 3) was isolated in the early 1970s from the Ethiopian plant, *Maytenus serrata* (Hochst. Ex A. Rich.) Wilczek (Celastraceae), again collected for the NCI through the USDA random collection program. The yields were very low ($2 \times 10^{-5}\%$ based on plant dry weight), but its extreme potency in testing against cancer cell lines permitted the production of sufficient limited quantities for pursuit of preclinical and clinical development. Despite very promising activity observed in preclinical animal testing, no significant efficacy was observed in clinical trials, and it was dropped from further study in the early 1980s. Related compounds, the ansamitocins, were subsequently isolated from a microbial source, the Actinomycete, *Actinosynnema pretiosum*, and this posed the question as to whether the maytansines are actually plant products, or are produced through an association between a microbial symbiont and the plant; this is a topic of continuing study. The microbial source of closely related compounds allows for easier production

of larger quantities of this class of compounds, and this factor, together with their extreme potency, has stimulated continued interest in pursuing their development. A derivative of maytansine, DM1, conjugated with a monoclonal antibody (mAb) targeting small-cell lung cancer cells, is being developed as huN901-DM1 by the U.S. company, ImmunoGen, Inc. and British Biotech for the treatment of small-cell lung cancer. Another conjugate, known as SB408075 or huC242-DM1 (also known as Cantuzumab Mertansine), produced by the coupling of DM1 to huC242, a mAb directed against the *mut1* epitope expressed in a range of cancers, including pancreatic, biliary, colorectal and gastric cancers, was being developed by Glaxo-SmithKline, and is currently in Phase I clinical trials in the USA. DM1 has also been conjugated to J591, a mAb targeting the prostate specific membrane antigen (PSMA), and is in clinical trials against prostate cancer.

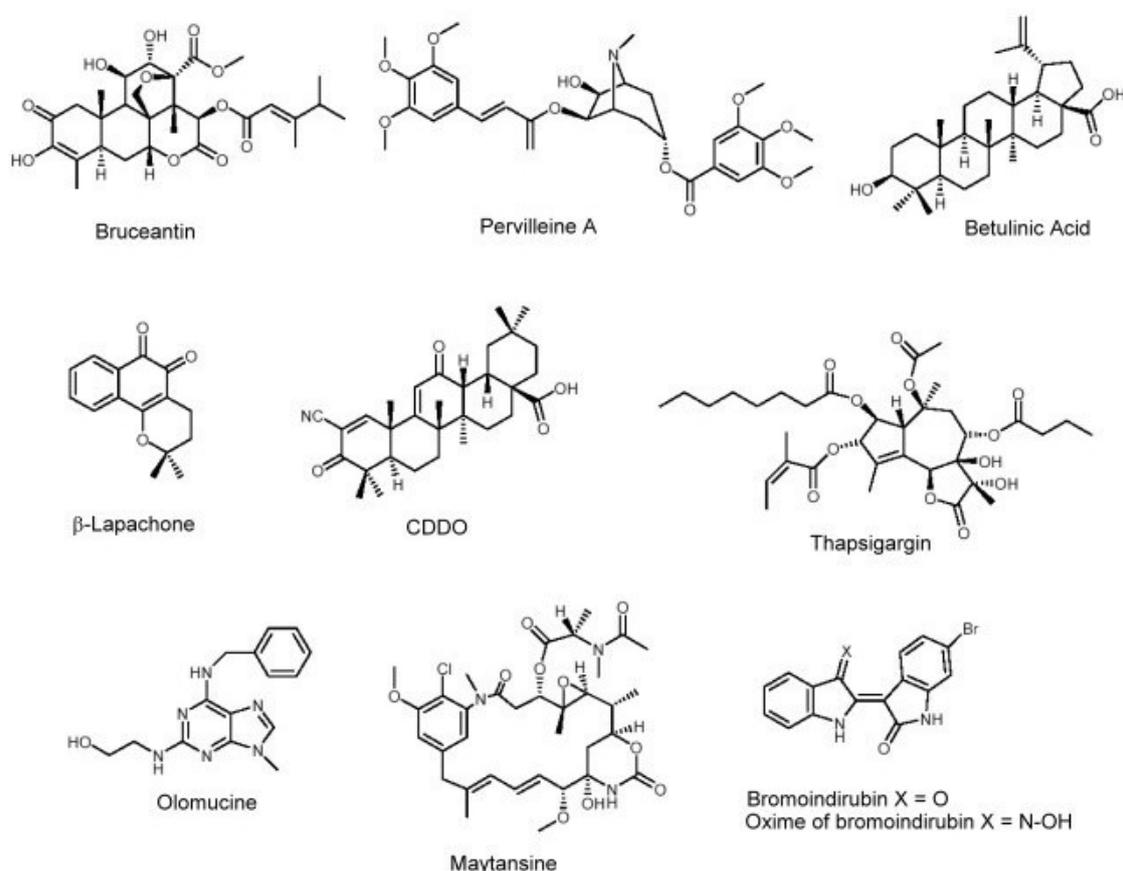


Figure 3. Plant-derived anti-tumor agents in preclinical development

Another case of considerable interest is that of thapsigargin (TG) (see Figure 3), isolated from the umbelliferous plant, *Thapsia garganica* L. (Apiaceae), collected on the Mediterranean island of Ibiza. Thapsigargin induces apoptosis (cell death) in quiescent and proliferating prostate cancer cells, and acts by disrupting intracellular Ca^{2+} levels. While TG does not show selectivity for prostate cancer cells, it has been conjugated to a small peptide carrier to produce a water-soluble prodrug which is specifically activated by prostate specific antigen (PSA) protease at metastatic prostate cancer sites. Treatment of animals bearing prostate cancer xenograft tumors demonstrated complete

tumor growth inhibition without significant toxicity. Given that the prodrug is stable in human plasma, it holds promise as a treatment for human prostate cancer, and it is being developed towards clinical trials with support from the NCI.

5. Plant-Derived Antitumor Agents in Preclinical Development (see Figure 3)

As mentioned above, a number of naturally-derived agents were entered into clinical trials and were terminated due to lack of efficacy or unacceptable toxicity. The case of maytansine (Section 3) illustrates how the emergence of novel technologies can revive interest in these “older” agents. It is also worth remembering that the development of effective drugs, such as paclitaxel (taxol[®]) and the camptothecin derivatives, topotecan and irinotecan (see Section 2), required 20 to 30 years of dedicated research and patience, and considerable resources, to ultimately prove their efficacy as clinical agents.

Another example of an “old” drug of the same vintage as taxol[®] and camptothecin, and having a possibility of revival, is bruceantin which was first isolated from a tree, *Brucea antidysenterica* J. F. Mill. (Simaroubaceae), used in Ethiopia for the treatment of “cancer”. Activity was observed in animal models bearing a range of tumors, but no objective responses were observed in clinical trials, and further development was terminated. Interest has been revived by the observation of significant activity against panels of leukemia, lymphoma and myeloma cell lines, as well as in animal models bearing early and advanced stages of the same cancers. This activity has been associated with the down-regulation of a key oncoprotein (*c-myc*), and these data are being presented as strong evidence supporting the development of bruceantin as an agent for the treatment of hematological malignancies.

Betulinic acid is a lupane-type triterpene which has been isolated from many taxonomically diverse plant genera. A major source is the birch tree, *Betula* spp. (Betulaceae), which is also a primary source of its C28 alcohol precursor, betulin. It is interesting to note that the isolation of betulin was first reported in 1788. Betulinic acid has been associated with a variety of biological activities, including antibacterial, anti-inflammatory and anti-malarial, but the most important activities have been associated with inhibition of the replication of strains of the human immunodeficiency virus (HIV), and cytotoxicity against a range of cancer cell lines. Significant *in vivo* activity has been observed in animal models bearing human melanoma xenografts, and the NCI is assisting in the development of systemic and topical formulations of the agent for potential clinical trials.

The family of bis-indoles known generically as indirubins are the main constituents of Mu Lan (*Indigofera tinctoria* L.), (Leguminosae) a product from the Chinese Materia Medica used to treat chronic myelogenous leukemia. The indirubins are the product of non-enzymatic dimerization of isatin and indoxyl—indole-derived molecules found in a large number of indigo-producing plants. These compounds are also produced by bacteria and are found in gastropod mollusks. In fact, in the latter case, they are the source of the purplish-red dye known from antiquity as “Tyrian Purple”. It is interesting to note that they were the first human-used compounds identified as inhibitors of cyclin dependent kinases (Cdks), key regulatory proteins in the cell cycle referred to in the

discussion of olomucine and roscovitine in Section 3 above. Other substituted indirubins have been synthesized, and the 3'-monooxime and 5-bromo derivative (Figure 3), show comparable activity to other known Cdk inhibitors, such as flavopiridol and roscovitine discussed earlier, and are candidates for preclinical development.

Triterpenoid acids, such as oleanolic and ursolic acid which are common plant constituents, are known to possess weak anti-inflammatory and anti-tumor activities. Attempts to synthesize new analogues with increased potencies have led to the synthesis of 2-cyano-3,12-dioxolean-1,9-dien-28-oic acid (CDDO) and its methyl ester, which have potent *in vitro* and *in vivo* anti-tumor activity against a wide range of tumors, including breast carcinomas, leukemias, and pancreatic carcinomas. Of particular interest is the significant activity of CDDO against epithelial ovarian carcinoma (EOC) cell lines, including lines which were resistant to clinically used agents such as cisplatin. Since EOC is the leading cause of death from gynecologic cancers, further evaluation of CDDO in the treatment of these cancers is being pursued.

Species of the genus *Tabebuia* (Bignoniaceae) have a history of use in the Amazonian region for the treatment of several diseases, including syphilis, fevers, malaria, cutaneous infections, and stomach disorders. Claims for clinical efficacy in the treatment of cancers started in the 1960s, particularly in Brazil, and this led to widespread sales of the stem bark and trunk wood of *T. impetiginosa* (Mart. Ex DC.) Standl. (synonym *T. avellanadae* Lorentz ex Griseb.), *T. rosea* (Bertol.), and *T. serratifolia* (Vahl) Nicholson in health food stores under various names such as pau d'arco or lapacho. They possess numerous bioactive compounds, but the naphthaquinones, particularly lapachol and β -lapachone, have received most attention. Lapachol showed significant *in vivo* anti-tumor activity in some early mouse models and was advanced to clinical trials by the NCI in the 1970s, but they were terminated due to unacceptable levels of toxicity. Recently, β -lapachone has stimulated renewed interest in this class of compounds due to its significant activity against a range of tumor cell lines, including breast, leukemia and prostate lines, and several multidrug resistant (MDR) lines. In addition, this class of compounds has been shown to be potent inhibitors of Cdc25 phosphatases, dephosphorylating enzymes that play a key role in cell cycle progression.

Consumption of *Veratrum californicum* by pregnant sheep has long been associated with the development of birth defects in lambs, including cyclopia in severe cases. These teratogenic effects have been found to be due to presence of alkaloids of the jervine class, in particular, cyclopamine, and are due to the specific inhibition of vertebrate cellular responses to the Hedgehog (Hh) family of secreted growth factors. While the Hedgehog cell signaling pathway normally is quiescent in adult cells, aberrant activation of the pathway in adults has been implicated in many cancers, including cancers of the pancreas, prostate, lung (small cell), and brain (glioma). Cyclopamine blocks the activation of this pathway, and analogues are in various stages of preclinical development.

The resistance developed by many cancer patients to treatment with standard anti-cancer agents is a serious problem encountered in cancer chemotherapy. Resistance to a drug may develop in a cell population through repeated exposure to treatment with that

particular drug, and this cell population may subsequently show broad cross-resistance to other anti-cancer agents even though it has never been exposed to those agents. This phenomenon is called multidrug resistance (MDR), and may be related to the presence of an MDR1 gene encoding a protein (Pgp; P-glycoprotein) which effectively pumps the drugs out of the cell, thereby precluding their anti-tumor actions. A good number of compounds which reverse this effect *in vitro* in cell line studies (so-called MDR inhibitors) have been discovered, but their effectiveness in the clinic has been disappointing in many cases. Thus, there is a continuing search for more effective MDR inhibitors. The pervilleines isolated from the Madagascar plant, *Erythroxylum pervillei* Baillon (Erythroxylaceae), have shown promising MDR activity both *in vitro* and *in vivo*, and pervilleine A is currently in preclinical development.

6. Cell Cycle Target Inhibition and Anticancer Drug Discovery

Up to the early 1990s, the discovery of novel anti-tumor agents from natural sources was largely based on testing for cytotoxic activity against cancer cell lines grown either *in vitro* or using *in vivo* models. Many of the naturally derived anti-cancer agents originally discovered using such assays, have been shown to exert their cytotoxic action through interaction with tubulin. Thus agents, such as vinblastine, vincristine, colchicine, combretastatin and maytansine promote the depolymerization of tubulin, while, in the case of the taxanes, microtubules are “bundled” as a result of stabilization against depolymerization. The discovery of Taxol[®] assumed an added measure of importance through the ground-breaking discovery of its unique mechanism of action. Although the taxanes are not considered ideal drug entities, they have been explored both therapeutically and chemically for many years while the search for more effective tubulin interactive agents has continued. This search has not been in vain, and a number of novel chemotypes which act by mechanisms similar to that of Taxol[®] have been discovered; these include two that, though reported as cytotoxins earlier, their actual biological mechanisms of action were not reported until 2004. One such plant-derived chemotype is the jatrophone esters, in particular jatrophone 1 from samples of the Corsican and Sardinian plant, *Euphorbia semiperfoliata* Viv. (Euphorbiaceae). Other recently discovered taxol mimics include the microbial metabolites, the epothilones, and the marine invertebrate metabolites, discodermolide, eleutherobin, sarcodictyins and the laulimalides.

Other important examples are the camptothecin derivatives, topotecan and irinotecan, which exert their cytotoxic action through inhibition of topoisomerase I, a fundamental enzyme complex involved in DNA “winding and unwinding”. In spite of significant efforts on the part of many research groups, few structural classes of compounds have demonstrated topoisomerase I inhibitory activity. Significant new classes of topoisomerase I inhibitors in preclinical development are the 2-aryl-quinoline derivatives (indenoquinolines), 3-aryl-isoquinoline derivatives (indeno-isoquinolines), and the naphthyridines which can be traced to the protoberberine alkaloids, such as nitidine, isolated from *Zanthoxylum* and *Fagara* species (Rutaceae).

With the identification of an increasing number of molecular targets associated with particular cancers, anti-cancer drug discovery is now based on high throughput screening of compounds against a range of such targets. Cyclin-dependent kinases

(Cdks), together with their cyclin partners, play a key role in the regulation of cell cycle progression, and inhibition of their activity delays or arrests progression at specific stages of the cell cycle. There are over 2000 kinases so far identified from genomic studies and all have a common site, the position where the ATP, i.e. the source of the phosphate that is donated, is bound.

An early example of a natural product compound class that ultimately led to Cdk inhibitors, was the moderately anti-tumor active flavonoid, quercetin. This flavanoid can be thought of as an ATP-mimic where the planar bicyclic chromone ring system is an isostere of adenine. Quercetin was shown to exert its anti-tumor effect through blocking cell cycle progression at the G0/G1 interface, consistent with Cdk inhibition; however, a close analogue, myricetin, shows an IC_{50} close to 10 μ M versus Cdk2. Flavopiridol (see Figure 1; section 2) showed about a 100 fold more selectivity for Cdks compared to its activity for tyrosine kinases, and was the first compound identified by the NCI as a potential anti-tumor agent that subsequently was proved to be a relatively specific Cdk inhibitor. Other examples mentioned above include olomucine and roscovitine (Section 3), and the indirubins (Section 5).

7. Conclusions

Plants have been a prime source of highly effective conventional drugs for the treatment of many forms of cancer. In many instances, the actual compound isolated from the plant may not serve as the drug, but leads to the development of potential novel agents. With the development of new technologies, some of the agents which failed earlier clinical studies are now stimulating renewed interest. The ability to attach agents to carrier molecules directed to specific tumors, holds promise for the effective targeting of highly cytotoxic natural products to the tumors while avoiding their toxic side effects on normal healthy tissues. With the rapid identification of new proteins having significant regulatory effects on tumor cell cycle progression, and their conversion into targets for high throughput screening, molecules isolated from plants and other natural organisms are proving to be an important source of novel inhibitors of the action of these key proteins, and have the potential for development into selective anti-cancer agents.

Glossary

Cell cycle:	A series of morphological and biochemical changes occurring from the initial phase of formation to the stage of cell division. See Bibliography, Newman et al., 2002, for details.
Clinical development:	Phase I studies are conducted to determine the maximum tolerated dose (MTD) of a drug in cancer patients, and to observe the sites and reversibilities of any toxic effects. Once the MTD has been determined and the clinicians are satisfied that no insurmountable problems exist with toxicities, the drug advances to Phase II clinical trials which test the efficacy of the drug against a range of different cancer disease types. In those cancers where significant responses are observed, Phase III trials are conducted to compare the activity of the drug with that of the best chemotherapeutic

agents currently available for the treatment of those cancers. In addition, the new drug may be tried in combination with other effective agents to determine if the efficacy of the combined regimen exceeds that of the individual drugs used alone.

Preclinical development:

The key steps are development of an adequate supply of the agent to permit pre-clinical and clinical development; formulation studies to develop a suitable vehicle to solubilize the drug for administration to patients, generally by intravenous injection or infusion in the case of cancer; pharmacological evaluation to determine the best route and schedule of administration to achieve optimal activity of the drug in animal models, the half-lives and bio-availability of the drug in blood and plasma, the rates of clearance and the routes of excretion, and the identity and rates of formation of possible metabolites; toxicological studies to determine the type and degree of major toxicities in rodent and dog models. These studies help to establish the safe starting doses for administration to human patients in clinical trials.

For a dictionary of cancer terms and cancer topics see <http://www.nci.nih.gov/dictionary/> and <http://www.nci.nih.gov/cancertopics/pdq>.

Bibliography

<http://www.cancerbacup.org.uk/Home>;

<http://www.cancerquest.org/>, and

<http://cancer.gov/Search/SearchClinicalTrialsAdvanced.aspx>. [These websites may be consulted for the latest information on the incidence and treatment of all forms of cancer].

Cassady J. M., Chan K. K., Floss H. G., and Leistner, E. (2004). Recent developments in the Maytansanoid anti-tumor agents. *Chem. Pharm. Bull.* **52**, 1-26 [This article describes the history and failure in early clinical trials of a potential anti-cancer agent, and the subsequent resurgence of interest with the development of new technologies].

Chen, J.K., Taipale, J., Cooper, M. K., and Beachy, P. A. (2002), Inhibition of hedgehog signaling by direct binding of cyclopamine to smoothened, *Genes Dev.*, **16**, 2743-2748. [This article discusses the role of hedgehog signaling in the cancer development pathway and the activity of cyclopamine in regulating this process]

Cichewitz R. H. and Kouzi S. A. (2004). Chemistry, biological activity, and chemotherapeutic potential of betulinic acid for the prevention and treatment of cancer and HIV infection. *Medical Research Reviews* **24**, 90-114. [A comprehensive review of recent developments with this ubiquitous plant-derived compound].

Cragg G. M., Boyd M. R., Cardellina II J. H., Newman D. J., Snader K. M., McCloud T. G.. (1994). Ethnobotany and drug discovery: the experience of the US National Cancer Institute. In *Ethnobotany and the Search for New Drugs. Ciba Foundation Symposium 185*, pp. 178-196. (eds. D. J. Chadwick and J. Marsh) Chichester, UK: Wiley & Sons. [This article reports that ethnomedical knowledge has not played a significant role in the NCI anti-cancer drug discovery program which has led the NCI to adopt broad ranging plant collections based on taxonomic diversity].

Cragg, G. M., Kingston, D. G. I., and Newman, D. J.. (Eds.) (2005) *Anticancer Agents from Natural Products* Boca Raton, Florida: Taylor & Francis. [This volume gives a comprehensive review of all the naturally-derived clinical agents in use or in clinical development for the treatment of cancer, including

chapters on the emaptothecins, combretastatins, homoharringtonine, maytansanoids, podophyllotoxins, taxanes, and the Vinca alkaloids].

Cuendet M. and Pezzuto J. M. (2004). Antitumor activity of Bruceantin. An old drug with new promise. *J. Nat. Prod.* **67**, 269-272. [This article illustrates the value of applying new insights and discoveries to the further study of a drug which initially was a clinical failure].

Hartwell J L. (1982). *Plants Used Against Cancer*. 709 pp. Lawrence, Massachusetts: [This book lists plants reported to possess anti-cancer activity, covering up to 1971. It has been updated by Farnsworth et al. (2000). *J. Ethnopharmacology*, **73**, 347-377. The term "cancer" may be poorly defined in many instances].

Kinghorn A. D. (1994). The discovery of drugs from higher plants. In *The Discovery of Natural Products with Therapeutic Potential*, 81-108. (ed. Gullo V. P.) Boston: Butterworth-Heinemann. [This article reviews plants as a source of drugs used in modern medicine].

Li, Q. and Sham, H. L. (2002). Discovery and development of antimetabolic agents that inhibit tubulin polymerisation for the treatment of cancer. *Expert Opin. Ther. Patents*. **12**, 1663-1701. [This article reviews many of the tubulin interactive anti-cancer agents, and includes a comprehensive discussion of compounds synthesized based on the combretastatin model].

Mi Q., Cui B., Silva G. L., Lantvit D., Reyes-Lim E., Chai H., Pezzuto J. M., Kinghorn A. D. and Swanson S. M. (2003). Pervilleine F, a new tropane alkaloid aromatic ester that reverses multidrug resistance. *Anticancer Research*, **23**, 3607-3616. [This article elaborates on the search for inhibitors of multidrug resistance].

Newman D. J., Cragg G. M. and Snader K. M. (2003). Natural products as sources of new drugs over the period 1981-2002. *Journal of Natural Products*, **66**, 1022-1037. [This article analyzes the currently used conventional drugs and their relationship to natural or synthetic origins].

Newman D. J., Cragg G. M., Holbeck S., and Sausville E. A. (2002). Natural products as leads to cell cycle pathway targets in cancer chemotherapy, *Current Cancer Drug Targets*, **2**, 279-308. [This article discusses the key role played by natural products as probes for cellular targets governing tumor cell cycle progression].

Biographical Sketches

Gordon Cragg was born in Cape Town, South Africa, and obtained his undergraduate training in chemistry at Rhodes University before proceeding to Oxford University where he obtained his D. Phil. in organic chemistry in 1963. After two years of postdoctoral research in natural products chemistry at the University of California, Los Angeles, he returned to South Africa to join the Council for Scientific and Industrial Research. In 1966, he was appointed to the staff of the Department of Chemistry at the University of South Africa, and transferred to the University of Cape Town in 1972. In 1979, he returned to the United States to join the Cancer Research Institute at Arizona State University, working with Professor G. Robert Pettit on the isolation of potential anti-cancer agents from plant and marine invertebrate sources. In 1985, he moved to the National Cancer Institute in Bethesda, Maryland, and was appointed Chief of the Natural Products Branch in 1989. His major interests lie in the discovery of novel natural product agents for the treatment of cancer and AIDS. He has been awarded the National Institutes of Health Merit Awards for his contributions to the development of the drug, taxol (1991), leadership in establishing international collaborative research in biodiversity and natural products discovery (2004), and contributions to developing and teaching NIH technology transfer training courses (2004). From 1998 to 1999 he served as President of the American Society of Pharmacognosy, and was elected to Honorary Membership of the Society in 2003. He has established collaborations between the National Cancer Institute and organizations in many countries promoting drug discovery from their natural resources. He has published over 100 papers related to these interests.

David Newman was born in Grays, Essex, UK. Initially he trained as a chemical analyst (Grad. RIC), followed by an M.Sc. in Organic Chemistry (University of Liverpool), and then after time in the UK chemical industry, he obtained a D.Phil. in Microbial Chemistry from the University of Sussex in 1968. Following two years of postdoctoral studies on the structure of electron transport proteins at the

University of Georgia, USA, he worked for Smith Kline and French in Philadelphia, Pennsylvania, as a biological chemist predominately in the area of antibiotic discovery. During this time period, he obtained an MS in Information Sciences in 1977 from Drexel University, Philadelphia. He has worked for a number of US-based pharmaceutical companies in natural products-based discovery programs in anti-infective and cancer treatments, and joined the Natural Products Branch of the NCI in 1991. He is responsible for the marine and microbial collection programs of the NCI, and in concert with Gordon Cragg, for the NCI's Open and Active Repository programs. In 2003 he was awarded the NIH Merit Award for his contributions to the development of potential anti-cancer agents from marine and microbial sources. His scientific interests are in the discovery and history of novel natural products as drug leads in the anti-infective and cancer areas, and in the application of information technologies to drug discovery. In conjunction with Gordon Cragg, he has established collaborations between the National Cancer Institute and organizations in many countries promoting drug discovery from their natural resources. He has published over 60 papers, presented over 60 abstracts, holds 17 patents that are related to these interests, is both a UK Chartered Chemist and a UK Chartered Biologist and is also an adjunct full professor at the Center of Marine Biotechnology, University of Maryland.

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