NATURAL PRODUCTS AS SOURCES OF ANTITUMOR AGENTS

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Contents

Summary

Naturally-derived compounds have played an important role in the development of over 60% of clinically useful anti-cancer agents, while a considerable number of natural products or analogs derived therefrom are in clinical and preclinical development. Brief discussions of some of these agents are presented, arranged according to the identity of their source organisms, and the significant role played by symbiotic microbes, such as endophytic fungi, in the production of agents originally thought to be derived from terrestrial plants or marine invertebrates is highlighted. In addition, the conjugation of toxic natural molecules to monoclonal antibodies or polymeric carriers, specifically targeted to epitopes on tumors of interest, as a means towards the development of efficacious targeted therapies is briefly discussed**.** While the mechanisms by which these agents exercise their antitumor action are mentioned, interested readers are referred to articles listed in the bibliography for more detailed discussions. The same

applies to readers interested in learning more about the increasingly important role played by microbial genomics in the drug discovery and development process.

1. Introduction

Throughout the ages humans have relied on nature for their basic needs and not least, medicines. Plants have formed the basis of sophisticated traditional medicine systems that have been in existence for thousands of years, and the first records, written on clay tablets in cuneiform, are from Mesopotamia and date from about 2600 BCE. Egyptian medicine dates from about 2900 BCE, with the best known Egyptian pharmaceutical record being the *Ebers Papyrus* dating from 1500 BCE. The Chinese *Materia Medica* has been extensively documented over the centuries, with the first record (Wu Shi Er Bing Fang), containing 52 prescriptions, dating from about 1100 BCE, though records from the *Pent'sao* are reputed to be even earlier (~2700 BCE); documentation of the Indian Ayurvedic system dates from about 1000 BCE (Susruta and Charaka).

The use of plants in the traditional medicine systems of many other cultures has been extensively documented. These plant-based systems continue to play an essential role in healthcare, and the World Health Organization has estimated by approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care. Plant products also play an important role in the health care systems of the remaining 20% of the population, mainly residing in developed countries. An analysis of data on prescriptions dispensed from community pharmacies in the United States from 1959 to 1980 indicated that about 25% contained plant extracts or active principles derived from higher plants and, at that time, at least 119 chemical substances, derived from 90 plant species, could be considered as important drugs in use in one or more countries. Of those 119 drugs, 74% were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine.

Plants have a long history of use in the treatment of cancer. Hartwell, in his review of plants used against cancer, lists more than 3,000 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 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. Despite these observations, however, it is significant that over 60% of currently used anticancer 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.

2. Antitumor Agents from Terrestrial Plant Sources

The search for anticancer 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. These discoveries prompted the United States National Cancer Institute (NCI) to initiate an extensive plant collection program in 1960, focused mainly in temperate regions, leading to the discovery of many novel chemotypes showing a range of cytotoxic activities.

These included 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, as yet, no new plant-derived clinical anticancer agents have reached the stage of general use. However, several novel leads have been discovered, and are various stages of preclinical development, as described in later sections.

2.1. Plant-Derived Anti-Cancer Agents in Clinical Use (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). The use of this plant by various cultures for the treatment of diabetes prompted its investigation as a source of potential oral hypoglycemic agents, but serendipitous observation of the reduction of white blood cell counts and bone marrow depression in rats led to the isolation of VLB and VCR.

More recent semi-synthetic analog of these agents are vinorelbine and vindesine. These agents act through the inhibition of tubulin polymerization and are primarily used in combination with other cancer chemotherapeutic drugs for the treatment of a variety of cancers, including leukemias, lymphomas, advanced testicular cancer, breast and lung cancers and Kaposi's sarcoma.

A long history of medicinal use, including the treatment of skin cancers and warts, led to the investigation of *Podophyllum peltatum* Linnaeus (commonly known as the American mandrake or Mayapple) (Podophyllaceae) as a source of potential antitumor agents. Podophyllotoxin was isolated as the active agent from the roots, and was also isolated from *P. emodii* Wallich, as species endemic to the Indian subcontinent.

Several closely related podophyllotoxin-like lignans were introduced into clinical trials, only to be dropped due to lack of efficacy and unacceptable toxicity. Extensive research, however, led to the development of the two clinically-active agents, etoposide and teniposide, which are semisynthetic derivatives of the natural product epipodophyllotoxin, an isomer of podophyltoxin.

These drugs are used in the treatment of lymphomas and bronchial and testicular cancers, and act through inhibition of topoisomerase II, an important enzyme involved in the replication pathway of DNA during cell cycle progression.

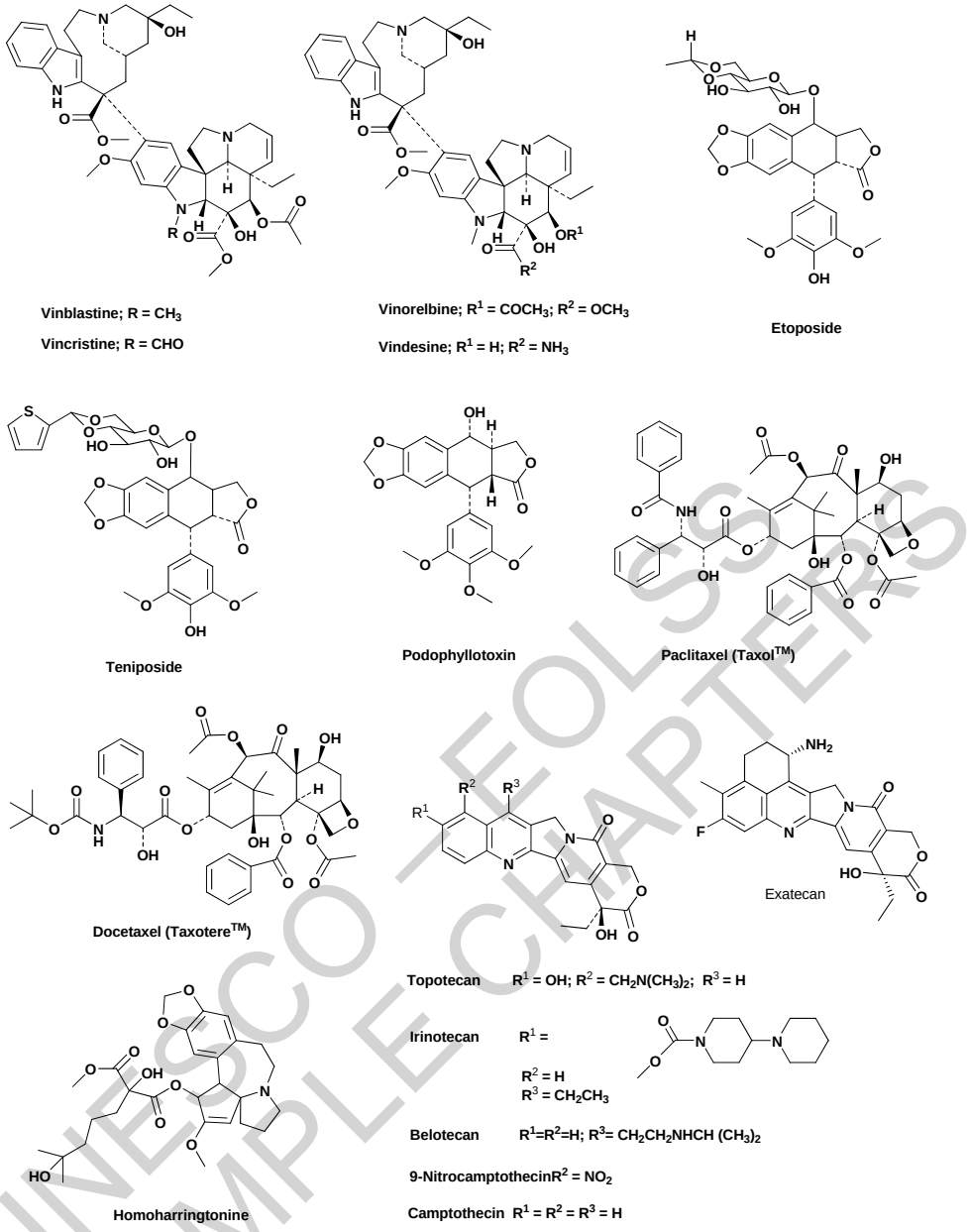

Figure 1. Plant-derived anti-cancer drugs in clinical use

More recent additions to the naturally-derived armamentarium of chemotherapeutic agents are the taxanes and camptothecins. Paclitaxel (Taxol®) initially was isolated from the bark of the Pacific yew, *Taxus brevifolia* Nutt. (Taxaceae), collected in Washington State, USA as part of a random collection program by the U.S. Department of Agriculture (USDA) for the NCI. While various parts of *T. brevifolia* and other *Taxus* species (e.g., *T. canadensis*, *T. baccata*) have reportedly been used by several Native American tribes for the treatment of some non-cancerous conditions, 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 analog, such as docetaxel (Taxotere®), has provided a major, renewable

natural source of this important class of drugs. The elucidation of their unique mechanism of action, involving the promotion of tubulin polymerization and stabilization of the resultant microtubules was, at the time, a landmark discovery in cancer chemotherapy. This led to intensive research aimed at the discovery of other chemotypes operating by a similar mechanism, and several of these discoveries are discussed in later sections of this chapter. Paclitaxel is used in the treatment of breast, ovarian and non-small cell lung cancer and has also shown efficacy against Kaposi's sarcoma, while docetaxel is primarily used in the treatment of breast cancer and non-small cell lung cancer. Paclitaxel has also attracted attention in the potential treatment of multiple sclerosis, psoriasis and rheumatoid arthritis, and as a constituent of stents. In addition, over 20 taxanes are in preclinical development as potential anticancer agents.

Camptothecin, isolated from the Chinese ornamental tree *Camptotheca acuminata* Decne, (Nyssaceae) was advanced (as its sodium salt) to clinical trials by NCI in the 1970s, but was dropped because of severe bladder toxicity. However, extensive research led to the development of the more effective semi-synthetic derivatives, topotecan (Hycamptin®), irinotecan (Camptosar®; CPT-11) and belotecan which are currently in clinical use. In addition, derivatives such as exatecan, 9-amino- and 9-nitro-camptothecin entered clinical trials but work has stopped on these earlier derivatives, though currently there are four to five different camptothecin derivatives in Phase I through Phase III clinical trials. These derivatives are variations on the basic camptothecin structure, with four or more variations of irinotecan encapsulated in various liposomal preparations, also in trials. Topotecan is used for the treatment of ovarian and small cell lung cancers, while irinotecan is used for the treatment of colorectal cancers and belotecan is also used for cervical carcinoma. This class of agents acts through inhibition of topoisomerase I, another important enzyme involved in the replication pathway of DNA during cell cycle progression and, to date, remains by far the most important class of topoisomerase I inhibitors.

Another plant-derived agent in clinical use is homoharringtonine, isolated from the Chinese tree *Cephalotaxus harringtonia* var. *drupacea* (Sieb and Zucc.) (Cephalotaxaceae). A racemic mixture of harringtonine and homoharringtonine has been used successfully in China for the treatment of acute myelogenous leukemia and chronic myelogenous leukemia. Purified homoharringtonine (omacetaxine mepesuccinate) has shown efficacy against various leukemias, including some resistant to standard treatment, and has been reported to produce complete hematologic remission in patients with late chronic phase chronic myelogenous leukemia.

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

Research by chemists at Hoechst India Ltd. in the early 1990s on *Dysoxylum binectariferum* Hook. f. (Meliaceae), phylogenetically related to the Ayurvedic plant, *D. malabaricum* Bedd., used for rheumatoid arthritis, led to the isolation of rohitukine as the constituent responsible for anti-inflammatory and immunomodulatory activity. A total synthesis was undertaken, and one of the over 100analogues 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, as well as broad spectrum *in vivo* activity against human tumor xenografts in mice. Flavopiridol was selected for preclinical and clinical development

and is currently in 19 Phase I and Phase II clinical trials, either alone or in combination with other anticancer agents, against a broad range of tumors, including leukemias, lymphomas and solid tumors. Added interest has been stimulated by observation of significant activity against chronic lymphocytic leukemia, a cancer currently lacking efficacious treatment.

Olomucine, originally isolated from the cotyledons of the radish, *Raphanus sativus* L. (Brassicaceae), shows weak inhibition of cyclin-dependent kinases, proteins which play a major role in cell cycle progression. The olomucine structural motif served as a model for the synthesis of roscovitine having greatly enhanced inhibitory activity, and which is currently in Phase II clinical trials in Europe as CYC202. Further combinatorial synthetic efforts based on the olomucine scaffold have led to the synthesis of the purvalanols which are even more potent, and to even more selective agents such as NU6140.

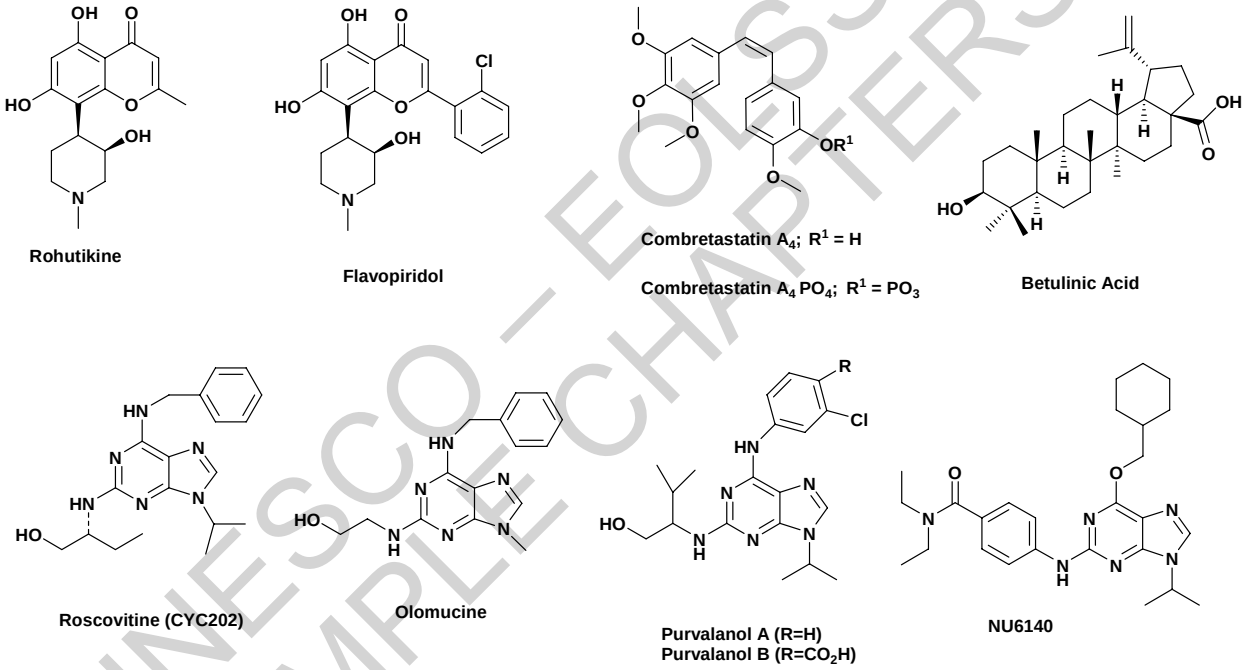

Figure 2. Plant-derived anti-cancer agents in clinical development

The combretastatins are a family of stilbenes isolated from the South African "bush willow" *Combretum caffrum* (Eckl. & Zeyh.) Kuntze (Combretaceae), collected in Southern Africa in the 1970s as part of a random collection program for the NCI by the USDA, working in collaboration with the Botanical Research Institute of South Africa. The combretastatins act as anti-angiogenic agents, causing vascular shutdown in tumors and resulting in tumor necrosis. A water-soluble analogue, combretastatin A-4 phosphate**,** has shown promise against thyroid cancer in early clinical trials. This chemical class has served as a model for the synthesis of a host of analogs containing the essential trimethoxy aryl moiety linked to substituted aromatic moieties through a variety of two or three atom bridges including heterocyclic rings and sulfonamides. It also provides an impressive display of the power of a relatively simple natural product structure to spawn a prolific output of medicinal and combinatorial chemistry. A

number of combretastatin mimics are being developed, and three analogues are in clinical trials while 11 are in preclinical development.

The lupane-type triterpene betulinic acid, isolated from many taxonomically diverse plant genera, is a plant-derived compound with a long history. A major source, the birch tree, *Betula* spp., is also a primary source of its C28 alcohol precursor, betulin, whose isolation of which was first reported in 1788. Cytotoxicity against a range of cancer cell lines coupled with significant *in vivo* activity in animal models bearing human melanoma xenografts, has resulted in the development of systemic and topical formulations, and currently an ointment is in a clinical trial for the treatment of dysplastic nevi (moderate to severe dysplasia). Additional biological activities, including antibacterial, anti-inflammatory and antimalarial, have been reported for betulinic acid and several derivatives, but the most important activities have been associated with inhibition of the replication of strains of the human immunodeficiency virus (HIV).

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approaches to identify symbiotic producers. In addition, strategies to isolate genes and gene clusters from marine species consortia are described].

Biographical Sketches

Gordon M. Cragg obtained his undergraduate training in chemistry at Rhodes University, South Africa, and his D. Phil. (organic chemistry) from Oxford University. After two years of postdoctoral research at the University of California, Los Angeles, he returned to S. Africa to join the Council for Scientific and Industrial Research. In 1966, he joined Chemistry Department at the University of South Africa, and transferred to the University of Cape Town in 1972. In 1979, he returned to the US to join the Cancer Research Institute at Arizona State University working with Professor G. R. Pettit. In 1985, he moved to the National Cancer Institute (NCI), National Institutes of Health (NIH) in Bethesda, Maryland, and was appointed Chief of the NCI Natural Products Branch in 1989. He retired in December, 2004, and is currently serving as an NIH Special Volunteer. His major interests lie in the discovery of novel natural product agents for the treatment of cancer and AIDS, with an emphasis on multidisciplinary and international collaboration. He has given over 100 invited talks at conferences in many countries worldwide, and has been awarded NIH Merit Awards for his contributions to the development of the anticancer drug, Taxol® (1991), leadership in establishing international collaborative research in biodiversity and natural products drug discovery (2004), and contributions to developing and teaching NIH technology transfer courses (2004). In 1998-1999 he was President of the American Society of Pharmacognosy and was elected to Honorary Membership of the Society in 2003, and was named as a Fellow of the Society in 2008. In November, 2006, he was awarded the "William L. Brown Award for Plant Genetic Resources" by Missouri Botanical Garden which also named a recently discovered Madagascar plant in his honor, *Ludia craggiana*, and in April, 2010, he was awarded an Honorary Doctorate of Science by his South African alma mater, Rhodes University. He has established collaborations between the NCI and organizations in many countries promoting drug discovery from their natural resources. He has published over 150 chapters and papers related to these interests.

David Newman is the current chief of the Natural Products Branch (NPB) in the Developmental Therapeutics Program at the National Cancer Institute in Frederick, MD. He was born in Grays, Essex UK in 1939. In 1963, he received an M.Sc. in synthetic organic chemistry from the University of Liverpool working under Prof. George Kenner, FRS on pyrrole and porphyrin syntheses. Following time as a synthetic chemist at Ilford, Ltd., he joined the ARC's Unit of Nitrogen Fixation at the University of London and then Sussex, as a research assistant in metallo-organic chemistry with Prof. J. Chatt, FRS., transferring to the microbial biochemistry group in early 1966 as a graduate student under Prof. John Postgate, FRS and was awarded a D. Phil. in 1968 for work on microbial electron transport proteins from *Desulfovibrio*. Following a move to the United States in Sept. 1968, he did two years as a post-doc at the Biochemistry Department at the University of Georgia working on protein sequencing of *Desulfovibrio* ferredoxins, and then in 1970 joined SK&F in Philadelphia as a biological chemist. At SK&F, most work was related to biological chemistry and antibiotic discovery and he left SK&F in 1985 when the antibiotic group was dissolved. For the next six years he worked in marine and microbial discovery programs (Air Products, SeaPharm and Lederle) and then in 1991, joined the NPB as a chemist responsible for marine and microbial collection programs. He was given the NIH Merit Award in 2003 for this work and following Gordon Cragg's retirement from the position of Chief, NPB at the end of 2004, he was acting chief until appointed chief in late 2006. He has been the author or coauthor of over 110 papers, reviews, book chapters (and an editor, with Gordon Cragg and David Kingston of *Anticancer Agents from Natural Products*), and holds18 patents.