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Discovery of new anticancer agents from higher plants

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1. ABSTRACT

Small organic molecules derived from higher plants have been one of the mainstays of cancer chemotherapy for approximately the past half a century. In the present review, selected single chemical entity natural products of plant origin and their semi-synthetic derivatives currently in clinical trials are featured as examples of new cancer chemotherapeutic drug candidates. Several more recently isolated compounds obtained from plants showing promising *in vivo* biological activity are also discussed in terms of their potential as anticancer agents, with many of these obtained from species that grow in tropical regions. Since extracts of only a relatively small proportion of the ca. 300,000 higher plants on earth have been screened biologically to date, bioactive compounds from plants should play an important role in future anticancer drug discovery efforts.

Keywords

Plants; Clinically used anticancer drugs; Compounds in clinical trials; Experimental agents with antineoplastic activity

2. INTRODUCTION

Natural products are generally defined as small-molecule secondary metabolites that originate from marine organisms, microorganisms, and plants. They occur as distinctive chemical types in taxonomically different organisms, for which they serve multiple biological functions related to organism survival. These naturally derived substances exhibit considerable structural diversity, and tend to adopt the preferred conformation and necessary steric complexity to exert varied activities in biological test systems. When compared with synthetic organic compounds as a whole, natural product molecules typically have more chiral centers, less hetero atoms, less heavy atoms, and more varied ring systems. Overall, natural products are generally regarded as possessing “drug-like” pharmacological qualities and “biologically friendly” molecular properties (1–3). These attributes make natural products an invaluable resource of chemical diversity and hence they have acted as excellent lead compounds for optimization by synthetic organic chemistry methods in anticancer agent discovery.

Natural products have proved to be very useful in anticancer chemotherapy drug development over last five decades, particularly those derived from terrestrial microbes and higher plants. An important review of the anticancer drugs introduced to the market in North America, western Europe, and Japan since the 1940s has indicated that some 47% of a total

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of the 155 anticancer drugs approved up to 2006 were either natural products or directly derived from natural product lead compounds by semi-synthesis (4). However, a reduced emphasis on natural products in terms of new drug development has become evident by large pharmaceutical companies during approximately the last 20 years (5–7). One of the major reasons underlying this decline is that the pharmaceutical industry has largely changed its strategy of drug discovery to the rapid high-throughput screening of molecular target-based pure compound chemical libraries, which have been generated particularly using combinatorial chemistry. Also, it is considered too time-consuming and labor-intensive to perform bioassay-guided isolation of natural products from crude extracts. Moreover, there is a tendency when screening natural product samples to rediscover bioactive compounds of already known structure. However, in recent years, new technologies have been developed to enhance natural product drug discovery in an industrial setting, including streamlined screening procedures and enhanced organism sourcing mechanisms (5–7). These changes actually bode well for the additional inclusion of natural product samples in anticancer drug discovery in the future. Indeed, several new anticancer agents of natural origin have been introduced to the market recently (8), and there is a promising pipeline of natural products in oncology-related clinical trials from various types of organisms (9–11).

The approved plant-derived oncology cancer chemotherapeutic agents used clinically in the United States may be structurally classified into four major groups: the vinca alkaloids, the epipodophyllotoxin lignans, the taxane diterpenoids, and the camptothecin quinoline alkaloid derivatives. The discoveries of the five antineoplastic lead compounds: vinblastine (vincaleukoblastine, **1**) (12, 13), vincristine (leurocristine, **2**) (13, 14), podophyllotoxin (**3**) (15) paclitaxel (taxol, **4**) (16), and camptothecin (**5**) (17), represent significant contributions to natural products isolation accomplished by pioneering natural product chemists, and these have all led subsequently to considerable advances in cancer chemotherapy. These compounds have been found to act on two important biochemical targets, tubulin and topoisomerase. According to our present knowledge, vinca alkaloids and taxanes both target cellular microtubulin, but they lead to cancer cell death through different specific mechanisms. Thus, vinca alkaloids such as vinblastine and vincristine bind to the microtubulin “vinca domain” site in the β -subunit, and disrupt the assembly of microtubules in mitosis (18), while paclitaxel acts as a microtubule stabilizer, by binding to the taxane site, and, as a result, interfering with the normal breakdown of microtubules during cell division (18, 19). Camptothecin and its clinically used analogues, irinotecan and topotecan, arrest the cell cycle at the S-phase by inhibiting the activity of topoisomerase I, leading to the inhibition of DNA replication and transcription (20, 21). Podophyllotoxin binds to tubulin and interferes with the formation of spindles in mitosis (22). In contrast, the two clinically used derivatives of podophyllotoxin, etoposide, and teniposide are topoisomerase II inhibitors, which arrest the cell cycle in the metaphase by stabilizing the covalent DNA-enzyme cleavable complex, and inducing topoisomerase II-mediated DNA breakage (22). Since there is already much published information on the above-mentioned four classes of plant-derived anticancer compounds (e.g., 23–25), these agents will not be further discussed in the present review.

Much of the progress in discovering and developing natural product drugs for the oncology market has been made by pharmaceutical and biotechnology companies. There has also been a long-standing commitment to this same end by the U.S. National Cancer Institute (NCI). Through the use of extramural and intramural funding mechanisms, the NCI has focused on the extensive collection and biological screening of extracts from plants and other organisms for their potential anticancer activity. The NCI has also played an important role in terms of antineoplastic drug development (26–29). During the period 1960 to 2004, some 174,000 plant samples were collected, including many from tropical regions, and these were screened through a systematic combination of *in vitro* and *in vivo* screens (25–27). In this process, extracts of small-scale plant samples were subjected to a standard solvent extraction protocol. Initially, the extracts were evaluated against a three cell-line panel, inclusive of MCF-7 breast, NCI-H460 lung, and SF-268 CNS cancer cells, with active samples then tested against a 60-cell line panel derived from leukemia and eight solid tumors including breast cancer, CNS tumor, colon cancer, non-small cell lung cancer, prostate cancer, renal cell cancer and ovarian cancer (25–27). Compounds of interest are then evaluated in the *in vivo* hollow fiber assay, a rapid and economic murine model developed at NCI in order to prioritize candidate compounds for possible further *in vivo* testing. In this assay, human cancer cells are grown within fibers that are implanted at subcutaneous or intraperitoneal sites in immunodeficient mice (30, 31). Further *in vivo* evaluation in murine xenograft systems is conducted for compounds deemed active in the *in vivo* hollow fiber assay (25–27).

In the following paragraphs of this review, a number of selected plant-derived antineoplastic single chemical entities currently under clinical trials as oncology drug candidates, and several promising lead compounds purified from plants, will be discussed in term of their plant origin, modes of action, as well as their potential use as anticancer agents.

3. SELECTED ANTINEOPLASTIC PLANT-DERIVED SINGLE CHEMICAL ENTITIES IN CLINICAL TRIALS

A relatively large number of chemical entities of plant origin are currently in clinical trials (9–11). However, a considerable percentage of the plant-derived oncology drug candidates in clinical trials are based on paclitaxel [ABI-007, DHA-paclitaxel, paclitaxel poliglumex, RPR-116278A, XRP9881 (RPR109881A)], camptothecin [9-aminocamptothecin, exatecan mesylate, oral topotecan (hycamtin), rubitecan (9-nitrocamptothecin, OrathecinTM)], vinblastine and vincristine (vinflunine ditartrate, vinorelbine, anhydrovinblastine, vincristine sulfate TCS), and epipodophyllotoxin (NK-611 and tafluposide 105) (9–11). Members of these already established classes of candidate drugs will not be discussed in this section,

with compounds of other structural types referred to instead. In this manner, it is hoped to better emphasize the overall structural diversity of promising chemical entities from plants as potential cancer chemotherapeutic agents.

Betulinic acid (**6**), a pentacyclic triterpenoid with a lupane skeleton, is widespread in the plant kingdom. This compound was purified as the active principle from *Ziziphus mauritiana* Lam. (Rhamnaceae), collected in Zimbabwe, using the bioassay-guided fractionation in earlier work by our group when at the University of Illinois at Chicago (32). In this investigation, betulinic acid was found to show *in vivo* selective growth inhibitory activity in athymic mice bearing human melanoma xenografts (32). This triterpenoid has been shown also to exhibit cytotoxicity against neuroectodermal and brain tumor cells (33). It induces apoptosis through regulation of the intrinsic pathway by changing mitochondrial membrane potential and activation of p38 MAPK and SAP/JNK by initiating ROS (reactive oxygen species) generation (34). This compound can be semi-synthesized by oxidation of betulin (**7**), a more abundant naturally occurring analogue (35). A betulinic acid-containing ointment is undergoing Phase I/II clinical evaluation for the treatment of dysplastic nevi with moderate to severe dysplasia (36).

Combretastatin A1 (**8**) and combretastatin A4 (**9**) are representative of several *cis*-stilbenes from *Combretum caffrum* Kuntze (Combretaceae), a shrub from South Africa, that were isolated in the 1980s by Pettit and colleagues at Arizona State University (37, 38). Substances in the combretastatin class are antineoplastic agents that also act as tubulin-binding and vascular disrupting agents (VDAs), and cause morphological changes within endothelial cells, which can lead to rapid and selective vascular collapse in solid tumors and result in the shutdown of the nutrient supply for malignant cells (39, 40). Combretastatin A4 phosphate (**10**, CA4P) is a phosphate prodrug of combretastatin A4 (**9**), which inhibits the polymerization of tubulin by binding on tubulin at the colchicine-site with highly affinity, and leads to the failure of microtubule formation (41). CA4P (**10**), both alone, and in combination with conventional oncological agents, is currently in several Phase I/II clinical trials for the treatment of anaplastic thyroid carcinoma and other advanced solid tumors in the United States (42, 43). AVE8062 (AC7700, **11**), a propanamide derivative of compound **9**, exhibits even a more potent antitumor effect than CA4P by inducing an irreversible blockage of tumor blood flow, and is now in Phase I clinical studies in Europe and the United States (44, 45). CA1P (Oxi4503, **12**), a bisphosphate prodrug of combretastatin A1 (**12**) also reported to be more potent than CA4P (**10**), is undergoing Phase I anticancer clinical trials in the U.K. (45).

Curcumin (**13**) is a phenolic diarylheptanoid isolated from turmeric, the roots of *Curcuma longa* L. (Zingiberaceae). Turmeric is a common spice widely consumed in many Asian countries, and has a long history of use in the traditional medicinal systems of India and China as a remedy for the treatment of ailments such as arthritis, digestive disorders, gallbladder and liver problems, and eye and skin infections (46, 47). The types of biological activities attributed to curcumin cover a wide range, and include antioxidant, anti-inflammatory, antimicrobial, immunomodulatory, and potential cancer chemopreventive effects (46, 47). Curcumin can induce apoptosis and inhibits the proliferation of a wide variety of malignant cells. The mechanisms underlying the activities exhibited by curcumin are complex, and involve the regulation of combined signaling pathways at multiple levels by acting on various targets. These include the modulation of gene transcription factors (NFkB, p53, AP-1), growth factors and their receptors (PDGF, EGF, VEGF), cell surface adhesion molecules (E-cadherin, β -cadenin), and protein kinases (CDKs, EGFR, PKC, p38 MAPK) (46, 47). Clinical studies of curcumin alone or in combination with other chemotherapeutic agents have been carried out in the United States and Israel for patients with colorectal and pancreatic cancers (48).

Flavopiridol (alvocidib, **14**) is a semisynthetic substance derived from rohitukine (**15**), an *N*-methylpiperidine alkaloid isolated initially from *Amoora rohituka* (Roxb.) Wight & Arn. (Meliaceae), and then identified as an anti-inflammatory and immunomodulatory agent from the stem bark of a native plant from India, *Dysoxylum binectariferum* Hiern (Meliaceae) (49, 50). Flavopiridol was found to exhibit cytotoxicity for a wide range of cancer cell lines and has demonstrated *in vivo* activity against prostate cancer, head and neck cancer, hematopoietic neoplasia, leukemia, and lymphoma xenograft murine models (51, 52). Mechanistic studies revealed that flavopiridol inhibits the activity of cyclin-dependent kinases (CDKs) by competing with ATP at their nucleotide binding sites, and causes cell cycle arrest at either the G₁ or G₁/M phases. Further studies indicated that flavopiridol also exhibits apoptosis induction, and anti-angiogenic and antiproliferative effects, by interacting at multiple other targets besides CDK (53, 54). Flavopiridol is the first cyclin-dependent kinase inhibitor in clinical trials for the treatment of patients with non-Hodgkin's lymphoma, renal, prostate, colon and gastric cancers (53–56).

Homoharringtonine (**16**, HHT), a cephalotaxine alkaloid ester, along with its analogue harringtonine (**17**), was first isolated in the early 1970s from the bark of *Cephalotaxus harringtonia* (Knight ex J. Forbes) K. Koch (Cephalotaxaceae), an evergreen tree native to East Asia, by Powell, Weisleder, and Smith at the United States Department of Agriculture facility at Peoria, Illinois (57). The only structural difference between these two alkaloids is that homoharringtonine possesses an additional methylene group in the ester side-chain when compared to harringtonine. Homoharringtonine (**16**) may be obtained also from several other *Cephalotaxus* species. The mechanism of action of compounds **16** and **17** is unusual, and they promote apoptosis and inhibit protein synthesis at the ribosomal level (58, 59). Clinical studies of a mixture of homoharringtonine (**16**) and harringtonine (**17**) were initiated in 1970s by the Chinese Academy of Medical Sciences, for use in the treatment of acute myeloid leukemia (AML) and chronic myeloid leukemia (CML). Homoharringtonine (**16**) has entered various Phase II/III clinical trials for patients with CML in the United States and in Europe (58–60). A semi-synthetic version of homoharringtonine (sHHT), also known as omacetaxine mepesuccinate and prepared by esterification of the parent compound cephalotaxine obtained from the leaves of a *Cephalotaxus* species, is undergoing a clinical trial for the treatment of CML in patients with imatinib resistance and intolerance (61).

Indigo (**18**) and indirubin (**19**) are two *bis*-indole alkaloids obtained from the blue dye “Indigo Naturalis”, which is also known as “Qing Dai” in the People's Republic of China. “Qing Dai” is a traditional Chinese medicinal herbal formula with antibacterial, anti-inflammatory, febrifuge, and hemostatic effects. It is composed of the dried residue derived from the leaves and/or stems of several plants that produce a dark blue dye, including *Baphicacanthus cusia* (Nees) Bremek. (Acanthaceae), *Indigofera suffruticosa* Mill. (Fabaceae), *Indigofera tinctoria* L. (Fabaceae), *Isatis tinctoria* L. (Brassicaceae), and *Polygonum tinctorium* Ait. (Polygonaceae) (62, 63). A mechanistic study has demonstrated that indirubin (**19**) exerts its antileukemic effect by competing with ATP for binding to the catalytic subunit of cyclin-dependent kinase (CDK), leading to the inhibition of this enzyme. The crystallized structure of a CDK2/indirubin analogue complex indicated an interaction with an ATP binding site via hydrogen bonds to key amino acids (64). Meisoindigo (1-methylisoindigo, **20**) is a derivative developed to improve the solubility in water and other pharmaceutical properties of indirubin. This compound showed significant activities against cancer cells through a multi-targeting profile including inhibition of DNA biosynthesis and assembly of microtubules, induction of cell differentiation, and down-regulation of c-myc gene expression (64, 65). Meisoindigo (**20**) has been subjected to clinical trial in the People's Republic of China for chronic myelogenous leukemia (CML) (66).

Lycopene (**21**), a well-known red pigment based on a β -carotene skeleton, is distributed widely in fruits and vegetables, and is especially abundant in tomatoes (*Solanum lycopersicum* L.; Solanaceae) and processed tomato products (67). The 40-carbon aliphatic chain of the molecule of lycopene contains thirteen *trans*- double bonds, with eleven of these being conjugated. Besides its antioxidant and anti-inflammatory activities, lycopene also exhibits anticarcinogenic properties in both *in vitro* and *in vivo* models (68, 69). Mechanism-of-action studies have indicated that lycopene exerts its anticancer and chemoprevention activities through the activation of the electrophile/antioxidant response element (EpRE/ARE) transcription system, inducing the expression of phase II detoxifying enzymes, and arresting the cell cycle at the G₀/G₁ phase by regulating cyclin D1 and the PI3K/Akt pathway (69). Lycopene has entered Phase II clinical trials in the United States for the prevention and treatment of prostate cancer (70).

2''-Oxovoruscharin (**22**), a cardenolide with a rare dihydrothiazole ring in its molecule, was isolated from a tropical evergreen shrub, *Calotropis procera* (Aiton) W.T. Aiton (Asclepiadaceae), and was demonstrated to have potent *in vitro* antitumor and Na⁺/K⁺-ATPase inhibitory activities (71). UNBS1450 (**23**), derived from 2''-oxovoruscharin by reducing the formyl group in the molecule to a hydroxymethyl group, exhibited an improved *in vitro* cytotoxicity profile activity when compared with the parent compound (72). A mechanistic investigation demonstrated that UNBS1450 induces the disruption of the actin cytoskeleton to affect multiple signaling pathways by binding to the sodium pump, and leads to non-apoptotic cell death (73). UNBS1450 has entered Phase I clinical studies in Europe for patients with solid tumors and lymphomas (74).

Perillyl alcohol (POH, **24**), is a monoterpene with a monocyclic carbon skeleton that is found in the essential oils in a number of plants such as lavender (*Lavendula x intermedia*;

Lamiaceae) and cherries [*Prunus avium* (L.) L. (Rosaceae)] (75). Preclinical studies have indicated that perillyl alcohol exhibits cytotoxicity for cancer cell lines derived from lung cancer, pancreatic cancer, prostate cancer, breast cancer and leukemia, and also showed *in vivo* inhibitory effects against UVB-induced skin carcinogenesis and DMBA-induced murine melanoma models (76, 77). Mechanistically, perillyl alcohol induces cell cycle arrest at the G₀/G₁ phase, by modulating the protein levels of cyclin-dependent kinases and cyclin-dependent kinase inhibitors (78). Perillyl alcohol is undergoing Phase I/II clinical trials in patients with breast cancer, ovarian cancer and glioblastoma multiform (79).

Protopanaxadiol [25; 20S-protopanaxadiol (PPD)] and protopanaxatriol [26; 20S-protopanaxatriol (PPT)] are two dammarane-type triterpenoids prepared by the hydrolysis of certain saponins obtained from Asian ginseng (*Panax ginseng* C.A. Mey.; Araliaceae) and related species (80). Protopanaxadiol induces cancer cell apoptosis and inhibits proliferation through targeting the Wnt/ β -catenin signaling pathway, down-regulating AKt activity, and inhibiting the effects of P-glycoprotein (P-gp) (81–83). Protopanaxadiol and protopanaxatriol are reported to have immunomodulating activity (84). A mixture of protopanaxadiol (25) and protopanaxatriol (26) (Pandimex™) has been approved conditionally in mainland China for the treatment of advanced cancers of the breast, colon-rectum, lung, and pancreas, and is ongoing a Phase I clinical trial in the United States for advanced lung, gastric, breast, and pancreatic cancers in combination with paclitaxel or alone (85, 86).

Resveratrol (27, 3,5,4'-trihydroxy-*trans*-stilbene) is a phenolic compound found in several dietary items, such as grapes (*Vitis vinifera* L.; Vitaceae), white mulberries (*Morus alba* L.; Moraceae), and peanuts (*Arachis hypogaea* L.; Fabaceae). This compound is also considered as a major constituent responsible for the cardioprotective activity of red wine (87, 88). Intensive studies have revealed that resveratrol possesses antioxidant, anti-inflammatory, and anticarcinogenic activities, and can prevent and slow a wide range of illnesses, including age-related diseases, cancer, cardiovascular problems, diabetes, and ischemic injuries (87–89). Resveratrol inhibits the growth of cancer cells and induces apoptosis by acting at multiple cellular targets, including activation of p53, inhibiting cyclooxygenase and cytochrome P450 enzymes, and activating AMP-activated kinase (AMPK) (87–89). Resveratrol has been also reported to show sensitization effects on drug-resistant tumor cells and to result in a synergistic cytotoxicity when combined with established anticancer therapies (90). This compound is now undergoing Phase I/II clinical trials for the prevention and treatment of colon cancer in the United States (91).

4. SOME PLANT-DERIVED COMPOUNDS WITH POTENTIAL ANTICANCER ACTIVITY

A series of anthracenone *C*-glycosides, alvaradoins E-N, was isolated from the leaves of *Alvaradoa haitiensis* Urb. (Picramniaceae), by Mansukh Wani and colleagues at Research Triangle Institute in North Carolina (92). Among these compounds, alvaradoin E (**28**), and its 10(*R*) isomer, alvaradoin F (**29**), were found to be the most potent cytotoxic agents against the KB cell line, exhibiting IC₅₀ values of 0.050 and 0.065 μM, respectively (92). Alvaradoins E and F were also evaluated in *in vivo* hollow fiber assay using KB, LNCaP, and Col2 cells, and both demonstrated significant inhibitory activity at the intraperitoneal site, while being less active when administered subcutaneously (93). In an *in vivo* P388 murine lymphocytic leukemia model study, alvaradoin E exhibited discernible activity (92). This compound was studied further for its mechanism of action. After treatment of LNCaP human prostate cancer cells with alvaradoin E, early signs of apoptosis including chromatin condensation and dose-dependent membrane depolarization were noticed. A significant decrease in cell viability accompanied by an increase in DNA breakage was observed using HL-60 leukemia cells (93).

Quassinoids are highly oxygenated degraded triterpenoid derivatives typically with a bitter taste, which are found in many species in the family Simaroubaceae. As the major active principles of these plants, quassinoids have been documented with a wide spectrum of biological activities, including anti-HIV, antimalarial, antiparasitic, antitumor, and insecticidal effects (94). Bruceantin (**30**), a quassinoid with potent cytotoxicity, was brought to clinical trials as an anticancer candidate by the U. S. NCI in the 1980s, but was eventually dropped because of a lack of demonstrated efficacy (95). Very recently, a new antitumor quassinoid, 2'-(*R*)-*O*-acetylglaucaurubinone (**31**) was isolated by Usami et al. from the bark of *Odyendyea gabonensis* (Pierre) Engler (96). Compound **31** exhibited potent cytotoxicity against a small panel of human cancer cell lines, including prostate (DU145), lung (A549), and oral epidermoid carcinoma (KB) cells, with ED₅₀ values around 0.05 μg/mL in each case. This compound was further evaluated using multiple breast cancer and ovarian cancer cell lines. Compound **31** demonstrated strong inhibitory effects against several ER- or/and PR-negative cell lines, suggesting that neither the estrogen receptor (ER) nor the progesterone receptor (PR) is the major target of this compound. Moreover, the HER2-overexpressing ER/PR-negative SKBR3 cell line was hypersensitive to this quassinoid, which implied that the HER2 signaling pathway might be involved in mediating its cytotoxicity (96). The *in vivo* mammary epithelial proliferation inhibitory activity of compound **31** was evaluated using a *Brcal/p53*-deficient mouse model. The mammary duct branching points were reduced to 32% after a daily intraperitoneal injection of 0.1/mg of compound **31** for one week, and the reduction effect on such branching was even more significant than observed for the control compounds, bruceantin (**30**) and paclitaxel (96). Further mechanism studies of action on this promising antineoplastic agent are being carried out.

More than 30 species in the family Amaryllidaceae have been documented as having a history of use as folk medicines for the treatment of tumors. Two isocarbostryl alkaloids, narciclasine (**32**) and pancratistatin (**33**), first isolated from bulbs of a *Narcissus* species (97) and from *Hymenocallis littoralis* Salisb. (formerly known as *Pancratium littorale* Jacq.) (98), respectively, are two of the most promising antineoplastic compounds from this plant family, and are effective and selective anticancer agents with the potential for clinical development. These two compounds exhibited *in vivo* growth inhibitory activity, in turn, in M5076 sarcoma (99) and P388 lymphocytic leukemia models (98). A study has indicated that normal human fibroblasts were nearly 250-fold less sensitive to narciclasine than MCF-7 cells and PC-3 cells (100). Pancratistatin selectively induced apoptosis of leukemia

cells from patients with minimal affect on normal peripheral blood mononuclear cells (101), and induced apoptosis specifically in breast cancer cells in comparison to non-cancerous cells (102). Pyridinium narciclasine (**34**) and sodium pancratistatin 3,4-*O*-cyclic phosphate (**35**) are prodrugs of narciclasine and pancratistatin, respectively, which have been produced to improve the water solubility of these two compounds (99, 103). Compound **35** was shown to retain the potency of cancer cell line inhibition when compared to the parent compound (**33**), and has been selected for preclinical development (103).

Tanshinone I (**36**), tanshinone IIA (**37**), and cryptotanshinone (**38**), are three major diterpene quinone derivatives isolated from the rhizomes of *Salvia miltiorrhiza* Bunge (Lamiaceae), which is known as “Tanshen” in traditional Chinese medicine and used as a herbal remedy with multiple therapeutic effects (104). These three tanshinone derivatives exhibited significant *in vitro* cytotoxicity against several human carcinoma cell lines (104). Tanshinone I (**36**) was found to inhibit the growth and invasion of breast cancer cells both *in vitro* and *in vivo* through regulation of adhesion molecules including ICAM-1 and VCAM-1 (105), and induce apoptosis of leukemia cells by interfering with the mitochondrial transmembrane potential ($\Delta\psi_m$), increasing the expression of Bax, as well as activating caspase-3 (106). Tanshinone IIA (**37**) has been reported to inhibit the growth of cervical cancer cells through disrupting the assembly of microtubules, and induces G₂/M phase arrest and apoptosis (107). This compound (**37**) can also inhibit invasion and metastasis of hepatocellular carcinoma (HCC) cells both *in vitro* and *in vivo*, by suppressing the expression of the metalloproteinases, MMP2 and MMP9 and interfering with the NF κ B signaling pathway (108). Cryptotanshinone (**38**) was reported to induce cell-cycle arrest at the G₁-G₀ phase, which was accompanied by the inhibition of cyclin D1 expression, retinoblastoma (Rb) protein phosphorylation, and of the rapamycin (mTOR) signaling pathway (109). Compound **38** can sensitize DU-345 breast cancer cells by suppressing the expression of the apoptosis inhibitory protein, Bcl-2 (110). Neo-tanshinlactone (**39**) is a new lead antineoplastic compound isolated from *S. miltiorrhiza* by Dr. Kuo-Hsiung Lee's research group at the University of North Carolina (111). Unlike the tanshinones, neo-tanshinlactone (**39**) possesses a lactone feature rather than an *ortho*-quinone moiety in ring C. This compound exhibited selectivity against two estrogen receptor-positive (ER⁺) breast cancer cell lines, MCF-7 and ZR-75-1, with ED₅₀ values, in turn, of 0.6 and 0.3 μ g/mL (111). Compound **40**, a synthetic analogue of neo-tanshinlactone (**39**), was found to inhibit the growth of the estrogen-dependent breast cancer cells, ZR-75-1, in an *in vivo* xenograft model (112). These investigations suggest that neo-tanshinlactone (**39**) and its derivatives might be promising candidates for the treatment of hormone-dependent breast cancers.

Silvestrol (**41**), and its 5''' *S* epimer, episilvestrol (**42**), were isolated by Kinghorn et al. from the tropical plant *Aglaia foveolata* Pannell (Meliaceae), with the structure and absolute configuration elucidated using single-crystal X-ray crystallography analysis (113). Silvestrol has also been documented as an antineoplastic constituent of *Aglaia leptantha*, collected in Sarawak, Malaysia, but with the stereochemistry of the 1,4-dioxanyloxy ring undetermined (114). This “flavagline” derivative was found to possess potent cytotoxicity for a small panel of human cancer cell lines and exhibited activity in both *in vivo* hollow fiber and P-388 lymphocytic leukemia assays (113). The total synthesis of silvestrol (**41**) has been accomplished by two independent groups (115–117). Preliminary mechanistic studies conducted by Dr. Steven Swanson and co-workers at the University of Illinois at Chicago showed that in LNCaP human prostate cancer cells, silvestrol produces a p53-independent cell-cycle blockage at the G₂/M check-point, and induces apoptosis through the regulation of caspases-2, -9 and -10 but not caspases-3 and -7 (118, 119). Additional investigation at The Ohio State University Medical Center, in the laboratory of Dr. Michael Grever, demonstrated that silvestrol exhibited B-cell selective activity and showed efficacy in both chronic lymphocytic leukemia and acute lymphocytic leukemia models (120). Silvestrol can

lead an early reduction in Mcl-1 expression in chronic lymphocytic leukemia cells (120), and inhibits the translation of malignancy-related mRNA by regulating initiation factor eIF4A (121). The compound demonstrated synergy with other agents using acute myelogenous leukemia cells (122) and modulated sensitivity to doxorubicin in a pre-clinical mouse lymphoma model (123). Further preclinical evaluation of silvestrol (**41**) as a potential antileukemic agent is ongoing.

5. CONCLUSION

Although cancer is one of the most pressing health concerns worldwide, the success rate in oncology new drug development is more than three times less than that for cardiovascular diseases (124). Cancer drug discovery is difficult because of many inherent obstacles such as tumor heterogeneity (124). Chemotherapy is an important option in modern cancer treatment, and plant-derived chemotherapeutic agents have contributed greatly to the progress of oncology chemotherapy development and to clinical practice. From the examples of antineoplastic candidates described in this review, it is evident that small molecules of plant origin continue to be valuable as sources of potential lead compounds in anticancer drug discovery. According to one recent estimate, more than 85% of higher plants have not been evaluated systematically for the presence of bioactive principles (125). There are more than 60 compounds from plant sources in the pipeline as potential anticancer agents (9–11). Innovations in the multidisciplinary investigative methods offer great promise for plant-derived drug discovery and development. These include new techniques related to compound isolation and structure elucidation, enhanced high-throughput biological screening procedures using novel biological targets, and continuing improvements in synthetic chemistry to make the optimization of the lead compounds more efficient. Therefore, there remains great potential to explore the plant kingdom for new antineoplastic

lead compound by using novel targets and newly developed technologies, so a promising future for anticancer drug development based on these agents can be predicted.

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References

1. Bindseil, Kai U.; Jakupovica, Jasmin; Wolf, Dietmar; Lavayre, Jacques; Leboul, Jean; van der Pyl, Didier. Pure compound libraries: a new perspective for natural product based drug discovery. *Drug Discov Today*. 2001; 6:840–847.10.1016/S1359-6446(01)01856-6 [PubMed: 11495757]
2. Firn, Richard D.; Jones, Clive G. Natural products - a simple model to explain chemical diversity. *Nat Prod Rep*. 2003; 20:382–391.10.1039/b208815k [PubMed: 12964834]
3. Vuorela P, Leinonen M, Saikku P, Tammela P, Rauha J-P, Wennberg T, Vuorela H. Natural products in the process of finding new drug candidates. *Curr Med Chem*. 2004; 11:1375–1389. [PubMed: 15180572]
4. Newman, David J.; Cragg, Gordon M. Natural products as sources of new drugs over the last 25 years. *J Nat Prod*. 2007; 70:461–477.10.1021/np068054v [PubMed: 17309302]
5. Koehn, Frank E.; Carter, Guy T. The evolving role of natural products in drug discovery. *Nature Rev Drug Disc*. 2005; 4:206–220.10.1038/nrd1657
6. Lam, Kin S. New aspects of natural products in drug discovery. *Trends Microbiol*. 2007; 15:279–289.10.1016/j.tim.2007.04.001 [PubMed: 17433686]
7. Li, Jesse W-H.; Vederas, John C. Drug discovery and natural products: end of an era or an endless frontier? *Science*. 2009; 325:161–165.10.1126/science.1168243 [PubMed: 19589993]
8. Bailly, Christian. Ready for a comeback of natural products in oncology. *Biochem Pharmacol*. 2009; 77:1447–1457.10.1016/j.bcp.2008.12.013 [PubMed: 19161987]
9. Saklani, Arvind; Kutty, Samuel K. Plant-derived compounds in clinical trials. *Drug Disc Today*. 2008; 13:161–171.10.1016/j.drudis.2007.10.010
10. Harvey, Alan L. Natural products in drug discovery. *Drug Disc Today*. 2008; 13:894–901.10.1016/j.drudis.2008.07.004
11. Butler, Mark S. Natural products to drugs: natural product-derived compounds in clinical trials. *Nat Prod Rep*. 2008; 25:475–516.10.1039/b514294f [PubMed: 18497896]
12. Noble RH, Cutts JH, Beer CH. Further biological activities of vincalkebostine – an alkaloid isolated from *Vinca rosea* (L.). *Biochem Pharmacol*. 1958:1347–1348.
13. Neuss, Norbert; Gorman, Marvin; Boaz, Harold E.; Cone, Nancy J. Vinca alkaloids. XI. Structures of leurocristine and vincalkebostine. *J Am Chem Soc*. 1962; 84:1509–1510.10.1021/ja00867a049
14. Svoboda, Gordon H. Alkaloids of *Vinca rosea* (*Catharanthus roseus*). IX. Extraction and characterization of leurosidine and leurocristine. *Lloydia*. 1961; 24:173–178.
15. Hartwell, Jonathan L.; Schrecker, Anthony W. Components of podophyllin. V. The constitution of podophyllotoxin. *J Am Chem Soc*. 1951; 73:2909–2916.10.1021/ja01150a143
16. Wani, Mansukh C.; Taylor, Harold Lawrence; Wall, Monroe E.; Coggon, Philip; McPhail, Andrew T. Plant antitumor agents. VI. Isolation and structure of taxol, a novel antileukemic and antitumor agent from *Taxus brevifolia*. *J Am Chem Soc*. 1971; 93:2325–2327.10.1021/ja00738a045 [PubMed: 5553076]
17. Wall, Monroe E.; Wani, MC.; Cook, CE.; Palmer, Keith H.; McPhail, AT.; Sim, GA. Plant antitumor agents. I. The isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. *J Am Chem Soc*. 1966; 88:3888–3890.10.1021/ja00968a057
18. Jordan, MaryAnn; Wilson, Leslie. Microtubules as a target for anticancer drugs. *Nat Rev*. 2004; 4:253–265.

19. Kingston, David GI. Tubulin-interactive natural products as anticancer agents. *J Nat Prod.* 2009; 72:507–515.10.1021/np800568j [PubMed: 19125622]
20. Cragg, Gordon M.; Newman, David J. A tale of two tumor targets: topoisomerase I and tubulin. the Wall and Wani contribution to cancer chemotherapy. *J Nat Prod.* 2004; 67:232–244.10.1021/np030420c [PubMed: 14987065]
21. Hsiang, Yaw-Huei; Lihou, Michelle G.; Liu, Leroy F. Arrest of replication forks by drug-stabilized topoisomerase I-DNA cleavable complex as a mechanism of cell killing by camptothecin. *Cancer Res.* 1989; 49:5077–5082. [PubMed: 2548710]
22. Hande KR. Etoposide: four decades of development of a topoisomerase II inhibitor. *Eur J Cancer.* 1998; 34:1514–1521.10.1016/S0959-8049(98)00228-7 [PubMed: 9893622]
23. Cragg, Gordon M.; Kingston, David GI.; Newman, David J. *Anticancer Agents from Natural Products.* CRC/Taylor & Francis; Boca Raton, FL: 2005.
24. Gordaliza M. Natural products as leads to anticancer drugs. *Clin Transl Oncol.* 2007; 9:767–776.10.1007/s12094-007-0138-9 [PubMed: 18158980]
25. Cragg, Gordon M.; Grothaus, Paul G.; Newman, David J. Impact on natural products on developing new anticancer agents. *Chem Rev.* 2009; 109:3012–3043.10.1021/cr900019j [PubMed: 19422222]
26. Suffness, Matthew; Douros, John. Current status of the NCI plant and animal program. *J Nat Prod.* 1982; 45:1–14.10.1021/np50019a001 [PubMed: 7069421]
27. Cragg, Gordon M.; Boyd, Michael R.; Khanna, Rita; Kneller, Robert; Mays, Thomas D.; Mazan, Kate D.; Newman, David J.; Sausville, Edward A. International collaboration in drug discovery and development: the NCI experience. *Pure Appl Chem.* 1999; 71:1619–1633.10.1351/pac199971091619
28. Cragg, Gordon M.; Newman, David J.; Yang, Stringner S. Natural product extracts of plant and marine origin having antileukemia potential. The NCI experience. *J Nat Prod.* 2006; 69:488–498.10.1021/np0581216 [PubMed: 16562862]
29. Hallock, Yali F.; Cragg, Gordon M. National Cooperative Drug Discovery Groups (NCDDGs): a successful model for public private partnerships in cancer drug discovery. *Pharm Biol.* 2003; 41(Suppl):78–91.10.1080/13880200390517779
30. Hollingshead, Melinda G.; Alley, Michael C.; Camalier, Richard F.; Abbott, Betty J.; Mayo, Joseph G.; Malspeis, Louis; Grever, Michael R. *In vivo* cultivation of tumor cells in hollow fibers. *Life Sci.* 1995; 57:131–141.10.1016/0024-3205(95)00254-4 [PubMed: 7603295]
31. Mi, Qiuwen; Pezzuto, John M.; Farnsworth, Norman R.; Wani, Mansukh C.; Douglas Kinghorn, A.; Swanson, Steven M. Use of the *in vivo* hollow fiber assay in natural products drug discovery. *J Nat Prod.* 2009; 72:573–580.10.1021/np800767a [PubMed: 19161316]
32. Pisha, Emily; Chai, Heebyung; Lee, Ik-Soo; Chagwedera, Tangai E.; Farnsworth, Norman R.; Cordell, Geoffrey A.; Beecher, Christopher WW.; Fong, Harry HS.; Douglas Kinghorn, A.; Brown, Daniel M.; Wani, Mansukh C.; Wall, Monroe E.; Heiken, Tina E.; Das Gupta, Tapas K.; Pezzuto, John M. Discovery of betulinic acid as a selective inhibitor of human melanoma that functions by induction of apoptosis. *Nat Med.* 1995; 1:1046–1051.10.1038/nm1095-1046 [PubMed: 7489361]
33. Zuco, Valentina; Supino, Rosanna; Righetti, Sabina C.; Cleris, Loredana; Marchesi, Edoardo; Gambacorti-Passerini, Carlo; Formelli, Franca. Selective cytotoxicity of betulinic acid on tumor cell lines, but not on normal cells. *Cancer Lett.* 2002; 175:17–25.10.1016/S0304-3835(01)00718-2 [PubMed: 11734332]
34. Laszczyk, Melanie N. Pentacyclic triterpenes of the lupane, oleanane and ursane group as tools in cancer therapy. *Planta Med.* 2009; 75:1549–1560.10.1055/s-0029-1186102 [PubMed: 19742422]
35. Sami, Alakurti; Tarua, Ma kela; Salmea, Koskimies; Jari, Yli-Kauhaluoma. Pharmacological properties of the ubiquitous natural product betulin. *Eur J Pharm Sci.* 2006; 29:1–13.10.1016/j.ejps.2006.04.006 [PubMed: 16716572]
36. U.S. National Institutes of Health. [accessed August 2010] Evaluation of 20% betulinic acid ointment for treatment of dysplastic nevi (moderate to severe dysplasia), 2010. Further information available at:

<http://www.cancer.gov/search/ViewClinicalTrials.aspx?cdrid=494341&version=HealthProfessional&protocolsearchid=3664931>

37. Pettit, George R.; Cragg, Gordon M.; Herald, Delbert L.; Schmidt, Jean M.; Lohavanijaya, Prasert. Isolation and structure of combretastatin. *Can J Chem.* 1982; 60:1374–1376.10.1139/v82-202
38. Pettit, George R.; Singh, Sheo Bux; Niven, Margaret L.; Hamel, Ernest; Schmidt, Jean M. Isolation, structure, and synthesis of combretastatins A-1 and B-1, potent new inhibitors of tubulin assembly, derived from *Combretum caffrum*. *J Nat Prod.* 1987; 50:119–131.10.1021/np50049a016 [PubMed: 3598594]
39. Young, Scott L.; Chaplin, David J. Combretastatin A4 phosphate: background and current clinical status. *Expert Opin Invest Drugs.* 2004; 13:171–1182.10.1517/13543784.13.9.1171
40. Siemann, Dietmar W.; Chaplin, David J.; Walicke, Patricia A. A review and update of the current status of the vasculature-disabling agent combretastatin-A4 phosphate (CA4P). *Expert Opin Investig Drugs.* 2009; 18:189–197.10.1517/13543780802691068
41. Pinney, Kevin G.; Jelinek, Christopher; Edvardsen, Klaus; Chaplin, David J.; Pettit, George R. The discovery and development of the combretastatins. In: Cragg, Gordon M.; Kingston, David GL.; Newman, David J., editors. *Anticancer Agents from Natural Products*. CRC Taylor & Francis; Boca Raton, FL: 2005. p. 23-46.
42. West, Catherine ML.; Price, Pat. Combretastatin A4 phosphate. *Anti-Cancer Drugs.* 2004; 5:179–187.10.1097/00001813-200403000-00001 [PubMed: 15014350]
43. ClinicalTrials.gov. [accessed October 2009] Study of Combretastatin and Paclitaxel/Carboplatin in the Treatment of Anaplastic Thyroid Cancer. Further information available at: <http://clinicaltrials.gov/ct2/show/NCT00507429>
44. Delmonte, Angelo; Sessa, Cristiana. AVE8062: a new combretastatin derivative vascular disrupting agent. *Expert Opin Invest Drugs.* 2009; 18:1541–1548.10.1517/13543780903213697
45. Lippert, John W, III. Vascular disrupting agents. *Bioorg Med Chem.* 2007; 5:605–615.10.1016/j.bmc.2006.10.020 [PubMed: 17070061]
46. Sa, Gaurisankar; Das, Tanya; Banerjee, Shuvomoy; Chakraborty, Juni. Curcumin: from exotic spice to modern anticancer drug. *Al Ameen J Med Sci.* 2010; 3:21–37.
47. Aggarwal, Bharat B.; Sundaram, Chitra; Malani, Nikita; Ichikawa, Haruyo. Curcumin: the Indian solid gold. *Adv Exp Med Biol.* 2007; 595:1–75. [PubMed: 17569205]
48. Dhillon, Navneet; Aggarwal, Bharat B.; Newman, Robert A.; Wolff, Robert A.; Kunnumakkara, Ajaikumar B.; Abbuzzese, James L.; Ng, Chaa S.; Badmaev, Vladimir; Kurzrock, Razelle. Phase II trial of curcumin in patients with advanced pancreatic cancer. *Clin Cancer Res.* 2008; 14:4491–4499.10.1158/1078-0432.CCR-08-0024 [PubMed: 18628464]
49. Harmon, Alan D.; Weiss, Ulrich; Silvertown, JV. The structure of rohitukine, the main alkaloid of *Amoora rohituka* (syn.*Aphanamixis polystachya*) (Meliaceae). *Tetrahedron Lett.* 1979; 20:721–724.10.1016/S0040-4039(01)93556-7
50. Lakdawala AD, Shirole MV, Mandrekar SS, Dohadwalla AN. Immunopharmacological potential of rohitukine: a novel compound isolated from the plant *Dysoxylum binectariferum*. *Asia Pac J Pharmacol.* 1988; 3:91–98.
51. Bible, Keith C.; Kaufmann, Scott H. Flavopiridol: a cytotoxic flavone that induces cell death in noncycling A549 human lung carcinoma cells. *Cancer Res.* 1996; 56:4856–4861. [PubMed: 8895733]
52. Arguello, Francisco; Alexander, Mark; Sterry, Judith A.; Tudor, Gabriela; Smith, Erik M.; Kalavar, Naina T.; Greene, John F., Jr; Koss, William; David Morgan, C.; Stinson, Sherman F.; Siford, Timothy J.; Gregory Alvord, W.; Klabansky, Richard L.; Sausville, Edward A. Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity *in vivo* against human leukemia and lymphoma xenografts. *Blood.* 1998; 91:2482–2490. [PubMed: 9516149]
53. Senderowicz, Adrian M. Flavopiridol: the first cyclin-dependent kinase inhibitor in human clinical trials. *Invest New Drugs.* 1999; 17:313–320.10.1023/A:1006353008903 [PubMed: 10665481]
54. Krystof, Vladimir; Uldrijan, Stjepan. Cyclin-dependent kinase inhibitors as anticancer drugs. *Curr Drug Targets.* 2010; 11:291–302.10.2174/138945010790711950 [PubMed: 20210754]

55. Schwartz, Gary K.; Ilson, David; Saltz, Leonard; O'Reilly, Eileen; Tong, William; Maslak, Peter; Werner, Jeanine; Perkins, Pam; Stoltz, Maxine; Kelsen, David. Phase II study of the cyclin-dependent kinase inhibitor flavopiridol administered to patients with advanced gastric carcinoma. *J Clin Oncol*. 2001; 19:1985–1992. [PubMed: 11283131]
56. Karp, Judith E.; Blackford, Amanda; Douglas Smith, B.; Alino, Katrina; Seung, Amy Hatfield; Bolanos-Meade, Javier; Greer, Jacqueline M.; Carraway, Hetty E.; Gore, Steven D.; Jones, Richard J.; Levis, Mark J.; McDevitt, Michael A.; Austin Doyle, L.; Wright, John J. Clinical activity of sequential flavopiridol, cytosine arabinoside, and mitoxantrone for adults with newly diagnosed, poor-risk acute myelogenous leukemia. *Leukemia Res*. 2010; 34:877–882.10.1016/j.leukres.2009.11.007 [PubMed: 19962759]
57. Powell RG, Weisleder D, Smith CR Jr. Antitumor alkaloids from *Cephalotaxus harringtonia*: structure and activity. *J Pharm Sci*. 1972; 61:1227–1230.10.1002/jps.2600610812 [PubMed: 5050371]
58. Itokawa, Hideji; Wang, Xihong; Lee, Kuo-Hsiung. Homoharringtonine and related compounds. In: Cragg, Gordon M.; Kingston, David GL.; Newman, David J., editors. *Anticancer Agents from Natural Products*. CRC/Taylor & Francis; Boca Raton, FL: 2005. p. 47-70.
59. Quintas-Cardama, Alfonso; Kantarjian, Hagop; Cortes, Jorge. Homoharringtonine, omacetaxine mepesuccinate, and chronic myeloid leukemia circa 2009. *Cancer*. 2009; 115:5382–5393.10.1002/cncr.24601 [PubMed: 19739234]
60. The Rocky Mountain Blood and Marrow Transplant Program (RMBMTP). [accessed October 2009] Initial patients treated in multinational Phase 2/3 study of ceflatonin 09-20-2006. 2009. Further information available at: <http://www.rockymountainbmt.com/news/Initial-Patients-Treated-in-Multinational-Phase-23-Study-of-Ceflatonin-4682.html>
61. Quintas-Cardama, Alfonso; Cortes, Jorge. Omacetaxine mepesuccinate - a semisynthetic formulation of the natural antitumoral alkaloid homoharringtonine, for chronic myelocytic leukemia and other myeloid malignancies. *IDrugs*. 2008; 11:356–372. [PubMed: 18465678]
62. Deng, Bailin. Direct colorimetric method for determination of indigo and indirubin in Qingdai. *Zhong Cao Yao*. 1986; 17:163–164.
63. Hoessel, Ralph; Leclerc, Sophie; Endicott, Jane A.; Nobel, Martin EM.; Lawrie, Alison; Tunnah, Paul; Leost, Maryse; Damiens, Eve; Marie, Dominique; Marko, Doris; Niederberger, Ellen; Tang, Weici; Eisenbrand, Gerhard; Meijer, Laurent. Indirubin, the active constituent of a Chinese antileukaemia medicine, inhibits cyclin-dependent kinases. *Nat Cell Biol*. 1999; 1:60–67. [PubMed: 10559866]
64. Xiao, Zhijian; Hao, Yushu; Liu, Bingcheng; Qian, Lingsheng. Indirubin and meisoindigo in the treatment of chronic myelogenous leukemia in China. *Leuk Lymph*. 2002; 43:1763–1768.10.1080/1042819021000006295
65. Zuo, Mingxin; Li, Yan; Wang, Hongbo; Zhou, Jianhua; Li, Hongyan; Liu, He; Xin, Hongqi; Zhang, Sen; Chen, Xiaoguo. The antitumor activity of meisoindigo against human colorectal cancer HT-29 cells *in vitro* and *in vivo*. *J Chemother*. 2008; 20:728–733. [PubMed: 19129071]
66. Cooperative Study Group of Phase III Clinical Trial on Meisoindigo. Phase II clinical trial on meisoindigo in the treatment of chronic myelogenous leukemia. *Zhonghua Xueyexue Zazhi*. 1997; 18:69–72. [PubMed: 15622780]
67. Kong, Kin-Weng; Khoo, Hock-Eng; Nagendra Prasad, K.; Ismail, Amin; Tan, Chin-Ping; Rajab, Nor Fadilah. Revealing the power of the natural red pigment lycopene. *Molecules*. 2010; 15:959–987.10.3390/molecules15020959 [PubMed: 20335956]
68. Sharoni, Yoav; Danilenko, Michael; Levy, Joseph. Molecular mechanisms for the anticancer activity of the carotenoid lycopene. *Drug Dev Res*. 2000; 50:448–456.10.1002/1098-2299(200007/08)50:3/4<448::AID-DDR28>3.0.CO;2-U
69. Bhuvaneswari V, Nagini S. Lycopene: A review of its potential as an anticancer agent. *Curr Med Chem- Anti-Cancer Agents*. 2005; 5:627–635.10.2174/156801105774574667
70. U.S. National Institutes of Health. [accessed August 2010] Lycopene in treating patients with metastatic prostate cancer. 2009. Further information available at <http://clinicaltrials.gov/ct2/show/NCT00068731>

71. Van Quaquebeke, Eric; Simon, Gentiane; Andre, Aurelie; Dewelle, Janique; El Yazidi, Mohamed; Bruyneel, Frederic; Tuti, Jerome; Nacoulma, Odile; Guissou, Pierre; Decaestecker, Christine; Braekman, Jean-Claude; Kiss, Robert; Darro, Francis. Identification of a novel cardenolide (2"-oxovoruscharin) from *Calotropis procera* and the hemisynthesis of novel derivatives displaying potent *in vitro* antitumor activities and high *in vivo* tolerance: structure-activity relationship analyses. *J Med Chem.* 2005; 48:849–856.10.1021/jm049405a [PubMed: 15689169]
72. Mijatovic, Tatjana; Lefranc, Florence; Van Quaquebeke, Eric; Van Vynckt, Frank; Darro, Francis; Kiss, Robert. UNBS1450: a new hemi-synthetic cardenolide with promising anti-cancer activity. *Drug Dev Res.* 2007; 68:164–173.10.1002/ddr.20178
73. Juncker, Tom; Schumacher, Marc; Dicato, Mario; Diederich, Marc. UNBS1450 from *Calotropis procera* as a regulator of signaling pathways involved in proliferation and cell death. *Biochem Pharmacol.* 2009; 78:1–10.10.1016/j.bcp.2009.01.018 [PubMed: 19447218]
74. Unibioscreen. [accessed August 2010] The pipeline – UNBS 1450. 2009. Further information available at http://www.unibioscreen.com/randd/pipeline_unbs1450.html
75. Belanger, James T. Perillyl alcohol: applications in oncology. *Altern Med Rev.* 1998; 3:448–457. [PubMed: 9855569]
76. Barthelman, Margaret; Chen, Weixing; Gensler, Helen L.; Huang, Chuanshu; Dong, Zigang; Tim Bowden, G. Inhibitory effects of perillyl alcohol on UVB - induced murine skin cancer and AP-1 transactivation. *Cancer Res.* 1998; 58:11–716.
77. Lluria-Prevatt, Maria; Morreale, Jeanne; Gregus, Jacie; Alberts, David S.; Kaper, Fiona; Giaccia, Amato; Powell, Marianne Broome. Effects of perillyl alcohol on melanoma in the TPas mouse model. *Cancer Epidemiol Biomark Prev.* 2002; 11:573–579.
78. Wiseman, Dean A.; Werner, Sean R.; Crowell, Pamela L. Cell cycle arrest by the isoprenoids perillyl alcohol, geraniol, and farnesol is mediated by p21Cip1 and p27Kip1 in human pancreatic adenocarcinoma cells. *J Pharmacol Exp Ther.* 2007; 320:1163–1170.10.1124/jpet.106.111666 [PubMed: 17138864]
79. da Gama Fischer, Juliana de Saldanha; Carvalho, Paulo Costa; da Costa Neves-Ferreira, Ana Gisele; da Fonseca, Clovts Orlando; Perales, Jonas; da Costa Carvalho, Maria da Gloria; Domont, Gilberto Barbosa. Anti-thrombin as a prognostic biomarker candidate for patients with recurrent glioblastoma multiform under treatment with perillyl alcohol. *J Exp Ther Oncol.* 2008; 7:285–290. [PubMed: 19227008]
80. Chu, Shi-Fen; Zhang, Jun-Tian. New achievements in ginseng research and its future prospects. *Chin J Integr Med.* 2009; 15:403–408.10.1007/s11655-009-0403-6 [PubMed: 20082243]
81. Popovich, David G.; Kitts, David D. Structure-function relationship exists for ginsenosides in reducing cell proliferation and inducing apoptosis in the human leukemia (THP-1) cell line. *Arch Biochem Biophys.* 2002; 406:1–8.10.1016/S0003-9861(02)00398-3 [PubMed: 12234484]
82. Li, Gang; Wang, Zhenhua; Sun, Yaxuan; Liu, Ke; Wang, Ziren. Ginsenoside 20(S)-protopanaxadiol inhibits the proliferation and invasion of human fibrosarcoma HT1080 cells. *Basic Clin Pharmacol Toxicol.* 2006; 98:588–592.10.1111/j.1742-7843.2006.pto_415.x [PubMed: 16700822]
83. Liu, Guo-Yu; Bu, Xuexian; Yan, Hang; William, W-G. Jia: 20S-Protopanaxadiol-induced programmed cell death in glioma cells through caspase-dependent and -independent pathways. *J Nat Prod.* 2007; 70:259–265.10.1021/np060313t [PubMed: 17261067]
84. Sun, Jianhua; Hu, Songhua; Song, Xiaoming. Adjuvant effects of protopanaxadiol and protopanaxatriol saponins from ginseng roots on the immune responses to ovalbumin in mice. *Vaccine.* 2007; 25:1114–1120.10.1016/j.vaccine.2006.09.054 [PubMed: 17069940]
85. Ouyang X, Yu Z, Chen Z, Xie F, Fang W, Peng Y, Chen X, Chen W, Wang W, Qi P, Jia W. A pilot study of safety and efficacy of Pandimex with or without paclitaxel in the treatment of advanced solid tumors. *J Clin Oncol.* 2005; 23:3188.
86. PanaGin Pharmaceuticals, Inc. [accessed August 2010] Clinical trials sponsored by pegasus pharmaceuticals group Inc. 2009. Further information available at <http://www.panagin.com/clinical.html>

87. Aggarwal, Bharat B.; Bhardwaj, Anjana; Aggarwal, Rishi S.; Seeram, Navindra P.; Shishodia, Shishir; Takada, Yasunari. Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. *Anticancer Res.* 2004; 24:2783–2840. [PubMed: 15517885]
88. Bishayee, Anupam; Politis, Themis; Darvesh, Altaf S. Resveratrol in the chemoprevention and treatment of hepatocellular carcinoma. *Cancer Treat Rev.* 2010; 36:43–53.10.1016/j.ctrv.2009.10.002 [PubMed: 19910122]
89. Athar, Mohammad; Back, Jung Ho; Tang, Xiuwei; Kim, Kwang Ho; Kopelovich, Levy; Bickers, David R.; Kim, Arianna L. Resveratrol: A review of preclinical studies for human cancer prevention. *Toxicol Appl Pharmacol.* 2007; 224:274–283.10.1016/j.taap.2006.12.025 [PubMed: 17306316]
90. Fulda, Simone; Debatin, Klaus-Michael. Sensitization for anticancer drug-induced apoptosis by the chemopreventive agent resveratrol. *Oncogene.* 2004; 23:6702–6711.10.1038/sj.onc.1207630 [PubMed: 15273734]
91. ClinicalTrials.gov. [accessed October 2010] Resveratrol for patients with colon cancer. 2009. Further information available at: <http://clinicaltrials.gov/ct2/show/NCT00256334>
92. Phifer, Sharnelle S.; Lee, Dongho; Seo, Eun-Kyoung; Kim, Nam-Cheol; Graf, Tyler N.; Kroll, David J.; Navarro, Hernan A.; Izydore, Robert A.; Jiménez, Francisco; Garcia, Ricardo; Rose, William C.; Fairchild, Craig R.; Wild, Robert; Soejarto, Djaja D.; Farnsworth, Norman R.; Douglas Kinghorn, A.; Oberlies, Nicholas H.; Wall, Monroe E.; Wani, Mansukh C. Alvaradoins E-N, antitumor and cytotoxic anthracenone C-glycosides from the leaves of *Alvaradoa haitiensis*. *J Nat Prod.* 2007; 70:954–961.10.1021/np070005a [PubMed: 17552563]
93. Mi, Qiuwen; Lantvit, Daniel; Reyes-Lim, Eulenia; Chai, Heebyung; Phifer, Sharnelle S.; Wani, Mansukh C.; Wall, Monroe E.; Tan, Ghee T.; Cordell, Geoffrey A.; Farnsworth, Norman R.; Douglas Kinghorn, A.; Pezzuto, John M. Apoptotic anticancer effect of alvaradoin E isolated from *Alvaradoa haitiensis*. *Anticancer Res.* 2005:779–787. [PubMed: 15868909]
94. Curcino Vieira, Ivo J.; Braz-Felho, Raimundo. Quassinoids Structural diversity, biological activity and synthetic studies. *Stud Nat Prod Chem.* 2006; 33(Part M):433–492.
95. Gragg, Gordon M.; Newman, David J. Plants as a source of anticancer agents. *J Ethnopharmacol.* 2005; 100:72–79.10.1016/j.jep.2005.05.011 [PubMed: 16009521]
96. Usami, Yoshihide; Nakagawa-Goto, Kyoko; Lang, Jing-Yu; Kim, Yoon; Lai, Chin-Yu; Goto, Masuo; Sakurai, Nobuko; Taniguchi, Masahiko; Akiyama, Toshiyuki; Morris-Natschke, Susan L.; Bastow, Kenneth F.; Cragg, Gordon; Newman, David J.; Fujitakeo, Mihoyo; Takeya, Koichi; Hung, Mien-Chie; Lee, Eva Y-HP.; Lee, Kuo-Hsiung. Antitumor Agents. 282. 2'-(R)-O-acetylglaucarubinone, a quassinoid from *Odyendyea gabonensis* as a potential anti-breast and anti-ovarian cancer agent. *J Nat Prod.* 2010; 73:1553–1558.10.1021/np100406d [PubMed: 20738103]
97. Ceriotti, Giovanni; Spandrio, Luigi; Gazzaniga, Annibale. Demonstration, isolation and physical and chemical characteristics of narciclasine, a new antimitotic agent of plant origin. *Tumori.* 1967; 53:359–371. [PubMed: 6069778]
98. Pettit, George R.; Gaddamidi, Venkatswamy; Cragg, Gordon M.; Herald, Delbert L.; Sagawa, Yoneo. Isolation and structure of pancratistatin. *J Chem Soc, Chem Commun.* 1984:1693–1694.10.1039/c39840001693
99. Pettit, George R.; Melody, Noeleen; Simpson, Michael; Thompson, Michael; Herald, Delbert L.; Knight, John C. Antineoplastic agents 500. Narcistatin. *J Nat Prod.* 2003; 66:92–96.10.1021/np020225i [PubMed: 12542352]
100. Dumont, Patrick; Ingrassia, Laurent; Rouzeau, Sebastien; Ribaucour, Fabrice; Thomas, Stephanie; Roland, Isabelle; Darro, Francis; Lefranc, Florence; Kiss, Robert. The Amaryllidaceae isocarbostryl narciclasine induces apoptosis by activation of the death receptor and/or mitochondrial pathways in cancer cells but not in normal fibroblasts. *Neoplasia.* 2007; 9:766–776.10.1593/neo.07535 [PubMed: 17898872]
101. Griffin, Carly; Hamm, Caroline; McNulty, James; Pandey, Siyaram. Pancratistatin induces apoptosis in clinical leukemia samples with minimal effect on non-cancerous peripheral blood mononuclear cells. *Cancer Cell Intern.* 2010; 10(6)10.1186/1475-2867-10-6
102. Siedlakowski, Peter; McLachlan-Burgess, Amanda; Griffin, Carly; Tirumalai, Sridhar S.; McNulty, James; Pandey, Siyaram. Synergy of pancratistatin and tamoxifen on breast cancer

cells in inducing apoptosis by targeting mitochondria. *Cancer Biol Ther.* 2008; 7:376–384.10.4161/cbt.7.3.5364 [PubMed: 18075307]

103. Pettit, George R.; Melody, Noeleen; Herald, Delbert L. Antineoplastic agents. 511. Direct phosphorylation of phenpanstatin and pancratistatin. *J Nat Prod.* 2004; 67:322–327.10.1021/np030299+ [PubMed: 15043403]
104. Wang, Xihong; Morris-Natschke, Susan L.; Lee, Kuo-Hsiung. New developments in the chemistry and biology of the bioactive constituents of Tanshen. *Med Res Rev.* 2007; 27:133–148.10.1002/med.20077 [PubMed: 16888751]
105. Nizamutdinova, Irina Tsoy; Lee, Gyeong Won; Lee, Jong Sil; Cho, Min Kyung; Son, Kun Ho; Jeon, Su Jin; Kang, Sam Sik; Kim, Yeong Shik; Lee, Jae Heun; Seo, Han Geuk; Chang, Ki Churl; Kim, Hye Jung. Tanshinone I suppresses growth and invasion of human breast cancer cells, MDA-MB-231, through regulation of adhesion molecules. *Carcinogenesis.* 2008; 29:1885–1892.10.1093/carcin/bgn151 [PubMed: 18586687]
106. Liu, Jia-Jun; Liu, Wen-Da; Yang, Hong-Zhi; Zhang, Yong; Fang, Zhi-Gang; Liu, Pei-Qing; Lin, Dong-Jun; Xiao, Ruo-Zhi; Hu, Yuan; Wang, Chun-Zhi; Li, Xu-Dong; He, Yi; Huang, Ren-Wei. Inactivation of PI3k/Akt signaling pathway and activation of caspase-3 are involved in tanshinone I-induced apoptosis in myeloid leukemia cells *in vitro*. *Ann Hematol.* 2010; 89:1089–1097.10.1007/s00277-010-0996-z [PubMed: 20512574]
107. Pan, Tai-Long; Hung, Yu-Chiang; Wang, Pei-Wen; Chen, Shui-Ten; Hsu, Teng-Kuei; Sintupisut, Nardnisa; Cheng, Chao-Sheng; Lyu, Ping-Chiang. Functional proteomic and structural insights into molecular targets related to the growth inhibitory effect of tanshinone IIA on HeLa cells. *Proteomics.* 2010; 10:914–929. [PubMed: 20049856]
108. Yuxian, Xu; Feng, Tian; Ren, Li; Zhengcai, Liu. Tanshinone II-A inhibits invasion and metastasis of human hepatocellular carcinoma cells *in vitro* and *in vivo*. *Tumori.* 2009; 95:789–795. [PubMed: 20210245]
109. Chen, Wenxing; Luo, Yan; Liu, Lei; Zhou, Hongyu; Xu, Baoshan; Han, Xiuzhen; Shen, Tao; Liu, Zhijun; Lu, Yin; Huang, Shile. Cryptotanshinone inhibits cancer cell proliferation by suppressing mammalian target of rapamycin-mediated cyclin D1 expression and Rb phosphorylation. *Cancer Prev Res.* 2010; 3:1015–1025.10.1158/1940-6207.CAPR-10-0020
110. Park, In-Ja; Kim, Min-Jung; Park, Ock Jin; Park, Myoung Gyu; Choe, Wonchae; Kang, Insug; Kim, Sung-Soo; Ha, Joohun. Cryptotanshinone sensitizes DU145 prostate cancer cells to Fas(APO1/CD95)-mediated apoptosis through Bcl-2 and MAPK regulation. *Cancer Lett.* 2010; 298:88–98.10.1016/j.canlet.2010.06.006 [PubMed: 20638780]
111. Wang, Xihong; Bastow, Kenneth F.; Sun, Chang-Ming; Lin, Yun-Lian; Hsi-Jung Yu, S.; Don, Ming-Jaw; Wu, Tian-Shung; Nakamura, Seikou; Lee, Kuo-Hsiung. Antitumor agents. 239. Isolation, structure elucidation, total synthesis, and anti-breast cancer activity of neo-tanshinlactone from *Salvia miltiorrhiza*. *J Med Chem.* 2004; 47:5816–5819.10.1021/jm040112r [PubMed: 15509181]
112. Dong, Yizhou; Shi, Qian; Pai, Huei-Chen; Peng, Chieh-Yu; Pan, Shiow-Lin; Teng, Che-Ming; Nakagawa-Goto, Kyoko; Yu, Donglei; Liu, Yi-Nan; Wu, Pei-Chi; Bastow, Kenneth F.; Morris-Natschke, Susan L.; Brossi, Arnold; Lang, Jing-Yu; Hsu, Jennifer L.; Hung, Mien-Chie; Lee, Eva Y-HP.; Lee, Kuo-Hsiung. Antitumor agents. 272. Structure-activity relationships and *in vivo* selective anti-breast cancer activity of novel neo-tanshinlactone analogues. *J Med Chem.* 2010; 53:2299–2308.10.1021/jm1000858 [PubMed: 20148565]
113. Hwang, Bang Yeon; Su, Bao-Ning; Chai, Heebyung; Mi, Qiuwen; Kardono, Leonardus BS.; Afriastini, Johar J.; Riswan, Soedarsono; Santarsiero, Bernard D.; Mesecar, Andrew D.; Wild, Robert; Fairchild, Craig R.; Vite, Gregory D.; Rose, William C.; Farnsworth, Norman R.; Cordell, Geoffrey A.; Pezzuto, John M.; Swanson, Steven M.; Douglas Kinghorn, A. Silvestrol and episilvestrol, potential anticancer rocaglate derivatives from *Aglaia silvestris*. *J Org Chem.* 2004; 69:3350–3358. *ibid.* 6156. [PubMed: 15132542]
114. Meurer-Grimes, Barbara Martha; Yu, Jin; Vairo, Gino Luigi. Therapeutic compounds and methods. US patent 6710075. 2004. p. B2
115. Gerard, Baudouin; Cencic, Regina; Pelletier, Jerry; Porco, John A. Enantioselective synthesis of the complex rocaglate (–)- silvestrol. *Angew Chem Int Ed.* 2007; 46:7831–7834.10.1002/anie.200702707

116. El Sous, Mariana; Khoo, Mui Ling; Holloway, Georgina; Owen, David; Scammells, Peter J.; Rizzacasa, Mark A. Total synthesis of (–)-episilvestrol and (–)-silvestrol. *Angew Chem Int Ed*. 2007; 46:7835–7838.10.1002/anie.200702700
117. Adams, Tim E.; El Sous, Mariana; Hawkins, Bill C.; Hirner, Sebastian; Holloway, Georgina; Khoo, Mui Ling; Owen, David J.; Paul Savage, G.; Scammells, Peter J.; Rizzacasa, Mark A. Total synthesis of the potent anticancer aglaia metabolites (–)-silvestrol and (–)-episilvestrol and the active analogue (–)-4'-desmethoxyepisilvestrol. *J Am Chem Soc*. 2009; 131:1607–1616.10.1021/ja808402e [PubMed: 19140688]
118. Mi, Qiuwen; Kim, Soyoung; Hwang, Bang Yeon; Su, Bao-Ning; Chai, Heebyung; Arbieva, Zarema H.; Douglas Kinghorn, A.; Swanson, Steven M. Silvestrol regulates G2/M checkpoint genes independent of p53 activity. *Anticancer Res*. 2006; 26:3349–3356. [PubMed: 17094452]
119. Kim, Soyoung; Hwang, Bang Yeon; Su, Bao-Ning; Chai, Heebyung; Mi, Qiuwen; Douglas Kinghorn, A.; Wild, Robert; Swanson, Steven M. Silvestrol, a potential anticancer rocaglate derivative from *Aglaia foveolata*, induces apoptosis in LNCaP cells through the mitochondrial/apoptosome pathway without activation of executioner caspase-3 or -7. *Anticancer Res*. 2007; 27:2175–2183. [PubMed: 17695501]
120. Lucas, David M.; Edwards, Ryan B.; Lozanski, Gerard; West, Derek A.; Shin, Jungook D.; Vargo, Melissa A.; Davis, Melanie E.; Rozewski, Darlene M.; Johnson, Amy J.; Su, Bao-Ning; Goettl, Virginia M.; Heerema, Nyla A.; Lin, Thomas S.; Lehman, Amy; Zhang, Xiaoli; Jarjoura, David; Newman, David J.; Byrd, John C.; Douglas Kinghorn, A.; Grever, Michael R. The novel plant-derived agent silvestrol has B-cell selective activity in chronic lymphocytic leukemia and acute lymphoblastic leukemia *in vitro* and *in vivo*. *Blood*. 2009; 113:4656–4666.10.1182/blood-2008-09-175430 [PubMed: 19190247]
121. Cencic, Regina; Carrier, Marilyn; Galicia-Vázquez, Gabriela; Bordeleau, Marie-Eve; Sukarieh, Rami; Bourdeau, Annie; Brem, Brigitte; Teodoro, Jose G.; Greger, Harald; Tremblay, Michel L.; Porco, John A., Jr; Pelletier, Jerry. Antitumor activity and mechanism of action of the cyclopenta[*b*]benzofuran, silvestrol. *PLoS ONE*. 2009; 4:e5223.10.1371/journal.pone.0005223 [PubMed: 19401772]
122. Cencica, Regina; Carrier, Marilyn; Trnkusa, Amanda; Porco, John A., Jr; Mindenc, Mark; Pelletier, Jerry. Synergistic effect of inhibiting translation initiation in combination with cytotoxic agents in acute myelogenous leukemia cells. *Leukemia Res*. 2010; 34:535–541.10.1016/j.leukres.2009.07.043 [PubMed: 19726085]
123. Bordeleau, Marie-Eve; Robert, Francis; Gerard, Baudouin; Lindqvist, Lisa; Chen, Samuel MH.; Wende, Hans-Guido; Brem, Brigitte; Greger, Harald; Lowe, Scott W.; Porco, John A., Jr; Pelletier, Jerry. Therapeutic suppression of translation initiation modulates chemosensitivity in a mouse lymphoma model. *J Clin Invest*. 2008; 118:1–11.
124. Kamb, Alexander; Wee, Susan; Lengauer, Christoph. Why is cancer drug discovery so difficult? *Nat Rev Drug Disc*. 2007; 6:115–120.10.1038/nrd2155
125. Pieters, Luc; Vlietinck, Arnold J. Bioguided isolation of pharmacologically active plant components, still a valuable strategy for the finding of new lead compounds? *J Ethnopharmacol*. 2005; 100:57–60.10.1016/j.jep.2005.05.029 [PubMed: 15996842]