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Minireview

Drug discovery from medicinal plants

Marcy J. Balunas [a], A. Douglas Kinghorn [b],*

[a] *Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, 833 South Wood Street, University of Illinois at Chicago, Chicago, IL 60612, USA*

[b] *Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA*

Abstract

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural product drugs of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials. As part of our National Cooperative Drug Discovery Group (NCDDG) research project, numerous compounds from tropical rainforest plant species with potential anticancer activity have been identified. Our group has also isolated several compounds, mainly from edible plant species or plants used as dietary supplements, that may act as chemopreventive agents. Although drug discovery from medicinal plants continues to provide an important source of new drug leads, numerous challenges are encountered including the procurement of plant materials, the selection and implementation of appropriate high-throughput screening bioassays, and the scale-up of active compounds.
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Keywords: Pharmacognosy; Medicinal plants; Natural products; National Cooperative Drug Discovery Group (NCDDG); Anticancer agents; Cancer chemoprevention; Challenges

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Introduction

Plants have been utilized as medicines for thousands of years (Samuelsson, 2004). These medicines initially took the form of crude drugs such as tinctures, teas, poultices, powders, and other herbal formulations (Balick and Cox, 1997; Samuelsson, 2004). The specific plants to be used and the methods of application for particular ailments were passed down through oral history. Eventually information regarding medicinal plants was recorded in herbals. In more recent history, the use of plants as medicines has involved the isolation of active compounds, beginning with the isolation of morphine from opium in the early 19th century (Kinghorn, 2001; Samuelsson, 2004). Drug discovery from medicinal plants led to the isolation of early drugs such as cocaine, codeine, digitoxin, and quinine, in addition to morphine, of which some are still in use (Newman et al., 2000; Butler, 2004; Samuelsson, 2004). Isolation and characterization of pharmacologically active compounds from medicinal plants continue today. More recently, drug discovery techniques have been applied to the standardization of herbal medicines, to elucidate

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analytical marker compounds. The following provides a brief review of the importance of medicinal plants in drug discovery including noteworthy compounds isolated from this source, our research involving anticancer and cancer chemopreventive drug discovery using medicinal plants, and finally current challenges in regard to medicinal plant drug discovery.

Drug discovery from medicinal plants has evolved to include numerous fields of inquiry and various methods of analysis. The process typically begins with a botanist, ethnobotanist, ethnopharmacologist, or plant ecologist who collects and identifies the plant(s) of interest. Collection may involve species with known biological activity for which active compound(s) have not been isolated (e.g., traditionally used herbal remedies) or may involve taxa collected randomly for a large screening program. It is necessary to respect the intellectual property rights of a given country where plant(s) of interest are collected (Baker et al., 1995). Phytochemists (natural product chemists) prepare extracts from the plant materials, subject these extracts to biological screening in pharmacologically relevant assays, and commence the process of isolation and characterization of the active compound(s) through bioassay-guided fractionation. Molecular biology has become essential to medicinal plant drug discovery through the determination and implementation of appropriate screening assays directed towards physiologically relevant molecular targets. Pharmacognosy encapsulates all of these fields into a distinct interdisciplinary science.

The definition and practice of pharmacognosy have been evolving since the term was first introduced about 200 years ago (Kinghorn, 2001; Samuelsson, 2004), as drug use from medicinal plants has progressed from the formulation of crude drugs to the isolation of active compounds in drug discovery. The American Society of Pharmacognosy refers to pharmacognosy as "the study of the physical, chemical, biochemical and biological properties of drugs, drug substances, or potential drugs or drug substances of natural origin as well as the search for new drugs from natural sources". As practiced today, pharmacognosy involves the broad study of natural products from various sources including plants, bacteria, fungi, and marine organisms. Pharmacognosy includes both the study of botanical dietary supplements, including herbal remedies (Tyler, 1999; Cardellina, 2002), as well as the search for single compound drug leads that may proceed through further development into Food and Drug Administration (FDA)-approved medicines. Drug discovery from medicinal plants is most frequently associated with the second of these two endeavors. Colleagues in Sweden have suggested a revised definition for pharmacognosy for these types of activities, namely as "a molecular science that explores naturally occurring structure–activity relationships with a drug potential" (Bruhn and Bohlin, 1997).

Importance of medicinal plants in drug discovery

Numerous methods have been utilized to acquire compounds for drug discovery including isolation from plants and other natural sources, synthetic chemistry, combinatorial chemistry, and molecular modeling (Ley and Baxendale, 2002; Geysen et al., 2003; Lombardino and Lowe, 2004). Despite the recent interest in molecular modeling, combinatorial chemistry, and other synthetic chemistry techniques by pharmaceutical companies and funding organizations, natural products, and particularly medicinal plants, remain an important source of new drugs, new drug leads, and new chemical entities (NCEs) (Newman et al., 2000, 2003; Butler, 2004). In both 2001 and 2002, approximately one quarter of the best-selling drugs worldwide were natural products or derived from natural products (Butler, 2004). There are also four new medicinal plant-derived drugs that have been recently introduced to the U.S. market (Fig. 1, **1–4**).

Arteether (**1**, trade name Artemotil®) is a potent anti-malarial drug and is derived from artemisinin, a sesquiterpene lactone isolated from *Artemisia annua* L. (Asteraceae), a plant used in traditional Chinese medicine (TCM) (van Agtmael et al., 1999; Graul, 2001). Other derivatives of artemisinin are in various stages of use or clinical trials as anti-malarial drugs in Europe (van Agtmael et al., 1999).

Galantamine (**2**, also known as galanthamine, trade name Reminyl®) is a natural product discovered through an ethnobotanical lead and first isolated from *Galanthus woronowii* Losinsk. (Amaryllidaceae) in Russia in the early 1950s (Heinrich and Teoh, 2004; Pirttila et al., 2004). Galantamine is
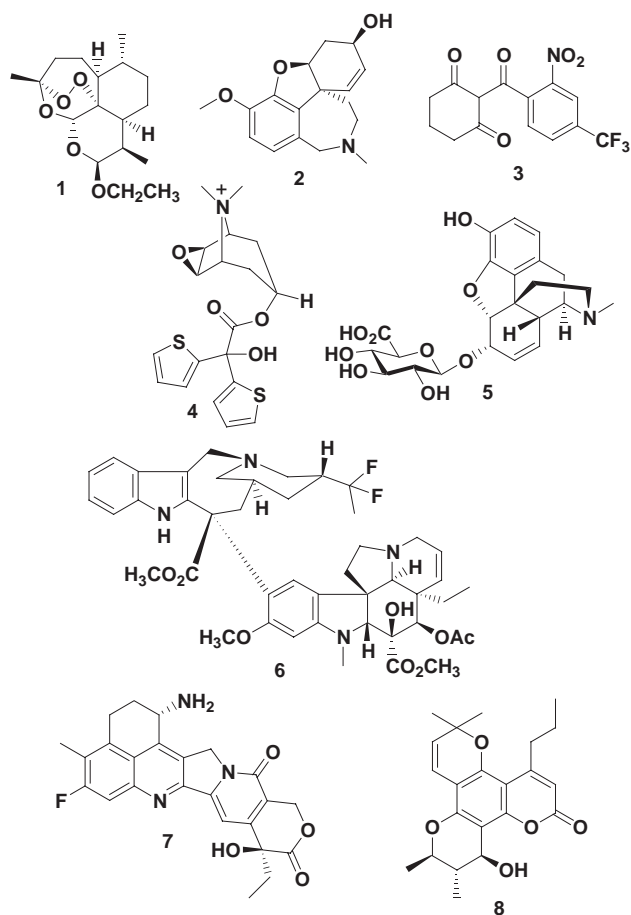

Fig. 1. Examples of new medicinal plant drugs recently introduced to market or in late-phase clinical trials.

approved for the treatment of Alzheimer's disease, slowing the process of neurological degeneration by inhibiting acetylcholinesterase (AChE) as well as binding to and modulating the nicotinic acetylcholine receptor (nAChR) (Heinrich and Teoh, 2004; Pirttila et al., 2004).

Nitisinone (**3**, trade name Orfadin®) is a newly released medicinal plant-derived drug that works on the rare inherited disease, tyrosinaemia, demonstrating the usefulness of natural products as lead structures (Frantz and Smith, 2003). Nitisinone is a modification of mesotrione, an herbicide based on the natural product leptospermone, a constituent of *Callistemon citrinus* Stapf. (Myrtaceae) (Hall et al., 2001b; Mitchell et al., 2001). All three of these triketones inhibit the same enzyme, 4-hydroxyphenylpyruvate dehydrogenase (HPPD), in both humans and maize (Hall et al., 2001b; Mitchell et al., 2001). Inhibition of the HPPD enzyme in maize acts as an herbicide and results in reduction of plastoquinone and tocopherol biosynthesis, while in humans the HPPD enzyme inhibition prevents tyrosine catabolism and the accumulation of toxic bioproducts in the liver and kidneys (Hall et al., 2001b).

Tiotropium (**4**, trade name Spiriva®) has recently been released to the United States market for treatment of chronic obstructive pulmonary disease (COPD) (Mundy and Kirkpatrick, 2004; Frantz, 2005). Tiotroprium is an inhaled anticholinergic bronchodilator, based on ipratropium, a derivative of atropine that has been isolated from *Atropa belladonna* L. (Solanaceae) and other members of the Solanaceae family (Barnes et al., 1995; Dewick, 2002; Mundy and Kirkpatrick, 2004). Tiotropium has shown increased efficacy and longer lasting effects when compared with other available COPD medications (Barnes, 2002; Mundy and Kirkpatrick, 2004).

Compounds **5**–**7** (Fig. 1) are all in Phase III clinical trials or registration and are subtle modifications of drugs currently in clinical use (Butler, 2004). M6G or morphine-6-glucuronide (**5**) is a metabolite of morphine from *Papaver somniferum* L. (Papaveraceae) and will be used as an alternate pain medication with fewer side effects than morphine (Lotsch and Geisslinger, 2001). Vinflunine (**6**) is a modification of vinblastine from *Catharanthus roseus* (L.) G. Don (Apocynaceae) for use as an anticancer agent with improved efficacy (Bonfil et al., 2002; Okouneva et al., 2003). Exatecan (**7**) is an analog of camptothecin from *Camptotheca acuminata* Decne. (Nyssaceae) and is being developed as an anticancer agent (Butler, 2004; Cragg and Newman, 2004). Modifications of existing natural products exemplify the importance of drug discovery from medicinal plants as NCEs and as possible new drug leads.

Calanolide A (**8**) (Fig. 1) is a dipyranocoumarin natural product isolated from *Calophyllum lanigerum* var. *austrocoriaceum* (Whitmore) P.F. Stevens (Clusiaceae), a Malaysian rainforest tree (Kashman et al., 1992; Yang et al., 2001; Yu et al., 2003). Calanolide A is an anti-HIV drug with a unique and specific mechanism of action as a non-nucleoside reverse transcriptase inhibitor (NNRTI) of type-1 HIV and is effective against AZT-resistant strains of HIV (Currens et al., 1996; Buckheit et al., 1999; Yu et al., 2003). Calanolide A is

currently undergoing Phase II clinical trials (Creagh et al., 2001).

Natural products have played an important role as new chemical entities (NCEs)—approximately 28% of NCEs between 1981 and 2002 were natural products or natural product-derived (Newman et al., 2003). Another 20% of NCEs during this time period were considered natural product mimics, meaning that the synthetic compound was derived from the study of natural products (Newman et al., 2003). Combining these categories, research on natural products accounts for approximately 48% of the NCEs reported from 1981–2002. Natural products provide a starting point for new synthetic compounds, with diverse structures and often with multiple stereocenters that can be challenging synthetically (Clardy and Walsh, 2004; Nicolaou and Snyder, 2004; Peterson and Overman, 2004; Koehn and Carter, 2005). Many structural features common to natural products (e.g., chiral centers, aromatic rings, complex ring systems, degree of molecule saturation, and number and ratio of heteroatoms) have been shown to be highly relevant to drug discovery efforts (Lee and Schneider, 2001; Feher and Schmidt, 2003; Clardy and Walsh, 2004; Piggott and Karuso, 2004; Koehn and Carter, 2005). Furthermore, since the escalation of interest in combinatorial chemistry and the subsequent realization that these compound libraries may not always be very diverse, many synthetic and medicinal chemists are exploring the creation of natural product and natural-product-like libraries that combine the structural features of natural products with the compound-generating potential of combinatorial chemistry (Hall et al., 2001a; Eldridge et al., 2002; Burke et al., 2004; Ganesan, 2004; Tan, 2004). Drugs derived from medicinal plants can serve not only as new drugs themselves but also as drug leads suitable for optimization by medicinal and synthetic chemists.

Even when new chemical structures are not found during drug discovery from medicinal plants, known compounds with new biological activity can provide important drug leads. Since the sequencing of the human genome, thousands of new molecular targets have been identified as important in various diseases (Kramer and Cohen, 2004). With the advent of high-throughput screening assays directed towards these targets, known compounds from medicinal plants may show promising and possibly selective activity. Several known compounds isolated from traditionally used medicinal plants have already been shown to act on newly validated molecular targets, as exemplified by indirubin, which selectively inhibits cyclin-dependent kinases (Hoessel et al., 1999; Eisenbrand et al., 2004) and kamebakaurin, which has been shown to inhibit NF-κB (Hwang et al., 2001; Lee et al., 2002). Other known compounds have also been shown to act on novel molecular targets, thus reviving interest in members of these frequently isolated plant compound classes. Three examples are cucurbitacin I, obtained from the National Cancer Institute (NCI) Diversity Set of known compounds and found to be highly selective in inhibiting the JAK/STAT3 pathway in tumors with activated STAT3 (Blaskovich et al., 2003), β-lapachone, which selectively kills cancer cells over normal cells through direct

checkpoint activation during the cell cycle (Li et al., 2003), and betulinic acid, with selective melanoma cytotoxicity through the activation of p38 (Pisha et al., 1995; Tan et al., 2003; Cichewicz and Kouzi, 2004).

Anti-cancer drug discovery

Worldwide, over ten million new cases of cancer (all sites excluding non-melanoma skin), with over six million deaths, were estimated in the year 2000 (Parkin, 2001; Parkin et al., 2001). Since 1990 there has been a 22% increase in cancer incidence and mortality with the four most frequent cancers being lung, breast, colorectal, and stomach and the four most deadly cancers being lung, stomach, liver, and colorectal (Parkin et al., 2001). Cancer is the second leading cause of death in the United States (U.S.), surpassed only by cardiovascular disease (Jemal et al., 2005). Although these figures are disquieting, some progress has been made in cancer diagnosis and treatment as evident through the high incidence of breast, prostate, testicular, and uterine cancers as compared with their relatively lower mortality (Parkin, 2001; Jatoi and Miller, 2003; Jemal et al., 2005).

Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (Newman et al., 2000, 2003; Butler, 2004). Of all available anticancer drugs between 1940 and 2002, 40% were natural products per se or natural product-derived with another 8% considered natural product mimics (Newman et al., 2003). Anticancer agents from plants currently in clinical use can be categorized into four main classes of compounds: vinca (or *Catharanthus*) alkaloids, epipodophyllotoxins, taxanes, and camptothecins. Vinblastine and vincristine were isolated from *Catharanthus roseus* (L.) G. Don (Apocynaceae) (formerly *Vinca rosea* L.) and have been used clinically for over 40 years (van Der Heijden et al., 2004). The vinca alkaloids and several of their semi-synthetic derivatives block mitosis with metaphase arrest by binding specifically to tubulin resulting in its depolymerization (Okouneva et al., 2003). Podophyllotoxin was isolated from the resin of *Podophyllum peltatum* L. (Berberidaceae) but was found to be too toxic in mice so derivatives were made with the first clinically approved drug being etoposide (Gordaliza et al., 2004). The epipodophyllotoxins bind tubulin, causing DNA strand breaks during the G_2 phase of the cell cycle by irreversibly inhibiting DNA topoisomerase II (Gordaliza et al., 2004). Paclitaxel was originally isolated from *Taxus brevifolia* Nutt. (Taxaceae) and was clinically introduced to the U.S. market in the early 1990s (Wall and Wani, 1996; Oberlies and Kroll, 2004). The taxanes, including paclitaxel and derivatives, act by binding tubulin without allowing depolymerization or interfering with tubulin assembly (Schiff et al., 1979; Horwitz, 2004). Camptothecin was isolated from *Camptotheca acuminata* Decne. (Nyssaceae) but originally showed unacceptable myelosuppression (Wall and Wani, 1996; Cragg and Newman, 2004). Interest in camptothecin was revived when it was found to act by selective inhibition of topoisomerase I,

involved in cleavage and reassembly of DNA (Cragg and Newman, 2004). Together, the taxanes and the camptothecins accounted for approximately one-third of the global anticancer market in 2002, over 2.75 billion dollars (Oberlies and Kroll, 2004). Numerous derivatives of all four compound classes have been synthesized, some of which are currently in clinical use. All of these natural products have led to significant biological discoveries related to their unique mechanisms of action.

With a National Cooperative Drug Discovery Group (NCDDG) grant funded by the U.S. National Cancer Institute (NCI) entitled "Novel Strategies for Plant-derived Anticancer Agents" (U01/U19 CA52956), we have endeavored to discover new anticancer agents from medicinal plants (see most recent review, Kinghorn et al., 2003). The current collaboration is now between The Ohio State University (OSU, responsible for isolation chemistry, dereplication, and the administrative core), University of Illinois at Chicago (UIC, responsible for plant selection, acquisition, and information management as well as in vitro and hollow fiber bioassays), Research Triangle Institute (RTI, a private research institute and a collaborator responsible for isolation chemistry and in vitro bioassays), and Bristol-Myers Squibb (BMS, our industrial collaborator that performs bioassays, lead optimization, and compound development), with a NCI program coordinator.

As of 2003, 5886 plant accessions had been collected representing 2582 species from 1358 genera in 288 families. Plant collections have occurred primarily in tropical forests due to their capacity to support highly diverse species with a large number of endemic taxa being threatened due to habitat loss (Burslem et al., 2001; Pitman and Jörgensen, 2002). A formal, written plant collection agreement is undertaken with source countries prior to all plant collections. Chloroform-soluble extracts are prepared from a small sample of each accession (Wall et al., 1996) and then screened in a battery of in vitro bioassays including a test panel of human tumor cell lines at UIC, as well as panels of diverse mechanistic and cell-based assays at RTI and BMS. Examples of mechanism-based assays include a proteasome inhibition assay (Almond and Cohen, 2002; Adams, 2004) and a histone deacetylase (HDAC) inhibition assay (Johnstone, 2002; Villar-Garea and Esteller, 2004). Active extracts are then subjected to LC−MS "dereplication" to determine if the extract contains previously isolated cytotoxic compounds (Cordell and Shin, 1999; Jones et al., 2003; Kinghorn et al., 2003). Extracts are prioritized for further phytochemical work if no masses of known cytotoxic compounds are found. Bioassay-guided fractionation is utilized to isolate and characterize the active compound(s), the most promising of which then undergo testing in the in vivo hollow fiber assay (Hollingshead et al., 1995; Mi et al., 2002). Further biological studies (e.g., mechanism of action and xenograft models) are performed to follow-up on compounds of significant interest.

Numerous types of bioactive compounds have been isolated as part of this NCDDG project including alkaloids, coumarins, cucurbitacins, diarylheptanoids, fatty acids, flavonoids, iridoids, lignans, limonoids, naphthoquinones, oligorhamnosides, physalins, phenanthrene derivatives, poly-

acetylenes, stilbenoids, sesquiterpenoids, and triterpenoids (Kinghorn et al., 1995, 1999, 2003; Kinghorn, 2001). Several of these compounds are currently undergoing further investigation (Fig. 2) including betulinic acid (**9**), pervilleine A (**10**), and silvestrol (**11**).

Betulinic acid (**9**), a pentacyclic triterpene, is a common secondary metabolite of plants, primarily from *Betula* species (Betulaceae). During the course of this NCDDG program, betulinic acid was isolated from *Ziziphus mauritiana* Lam. (Rhamnaceae) collected in Zimbabwe (Pisha et al., 1995). The ethyl acetate-soluble extract displayed selective cytotoxicity against human melanoma cells (MEL-2). Betulinic acid was isolated using bioassay-guided fractionation including silica gel chromatography and crystallization techniques. This compound was selectively cytotoxic against several human melanoma cancer cell lines (MEL-1 half-maximal effective dose (ED_{50}) = 1.1 µg/ml, MEL-2 ED_{50} = 2.0 µg/ml, and MEL-4 ED_{50} = 4.8 µg/ml). Betulinic acid was then found to be active in vivo using athymic mice carrying human melanomas, with little toxicity. Further biological studies indicated that betulinic acid works by induction of apoptosis (Pisha et al., 1995). Pre-clinical development towards a topical formulation is ongoing, spearheaded by Dr. Tapas K. Das Gupta of the University of Illinois at Chicago.

Pervilleine A (**10**), along with eight other tropane alkaloids, was isolated from the roots of *Erythroxylum pervillei* Baill. (Erythroxylaceae) collected in southern Madagascar (Silva et al., 2001). The chloroform-soluble extract was found to be selectively cytotoxic against a multi-drug resistant (MDR) oral epidermoid cancer cell line (KB-V1) in the presence of the anticancer agent vinblastine. The pervilleines were isolated using bioassay-guided fractionation including silica gel chromatography and aluminum oxide chromatography. Pervilleine

A was selectively cytotoxic against KB-V1 in the presence of vinblastine (ED_{50} = 0.3 µg/ml). Further in vitro studies related to the MDR inhibition were undertaken in comparison with existing MDR agents including verapamil (Mi et al., 2001). Pervilleine A was then tested in the in vivo hollow fiber model with promising results indicating that with KB-8-5 cells pervilleine A may be more effective than verapamil for reversing MDR (Mi et al., 2001). Further in vivo evaluation is planned for pervilleine A, including testing in a xenograft mouse model for MDR.

Silvestrol (**11**) was first isolated from the fruits of *Aglaia sylvestris* (M. Roemer) Merrill (Meliaceae) (later re-identified as *Aglaia foveolata* Pannell) collected in Indonesia (Hwang et al., 2004). The chloroform-soluble extract was found to be cytotoxic to several human cancer cell lines and, more importantly, the extract was active in the P-388 in vivo test system. Bioassay-guided fractionation was performed using silica gel chromatography and reversed-phase high-pressure liquid chromatography (HPLC) leading to the isolation of silvestrol. Silvestrol was cytotoxic against lung (Lu1, ED_{50} = 1.2 nM), prostate (LNCaP, ED_{50} = 1.5 nM), and breast (MCF-7, ED_{50} = 1.5 nM) cancer cells as well as against umbilical vein endothelial cells (HUVEC, ED_{50} = 4.6 nM). Silvestrol was then tested in the in vivo hollow fiber bioassay and exhibited dose-dependent cytotoxicity with no significant weight loss. Silvestrol also showed activity at a maximum tolerated dose of 2.5 mg/kg/injection when administered intraperitoneally twice daily for 5 days in the P-388 murine leukemia model (Hwang et al., 2004). Biological studies are ongoing to determine the mechanism(s) of action for silvestrol. Following recollection of the plant materials and subsequent re-isolation, silvestrol, or one of its analogs, will be subjected to further studies and hopefully pre-clinical development.

Two of these compounds, betulinic acid and pervilleine A, are currently being developed under the Rapid Access to Intervention Development (RAID) program at NCI which facilitates translation to the clinic of important therapeutics originating in the academic community (see http://dtp.nci/ nih.gov/docs/raid/raid_pp.html). Further studies on silvestrol are currently ongoing through the auspices of our NCDDG project.

Drug discovery for cancer chemoprevention

Still a relatively new field, cancer chemoprevention was first defined as "a strategy of cancer control by administration of synthetic or natural compounds to reverse or suppress the process of carcinogenesis" (Sporn et al., 1976). Carcinogenesis is a multistage process by which a normal cell is transformed into a cancerous cell. Transformation involves initiation, typically from DNA damaging agents, promotion, during which cell proliferation is increased, and progression, involving additional genetic alterations. Chemoprevention strategies target each of these steps including anti-initiation strategies (e.g., DNA repair, detoxification, free-radical scavenging, and carcinogen metabolism) and anti-promotion/anti-progression strategies (e.g., free-radical scavenging, proliferation suppres-
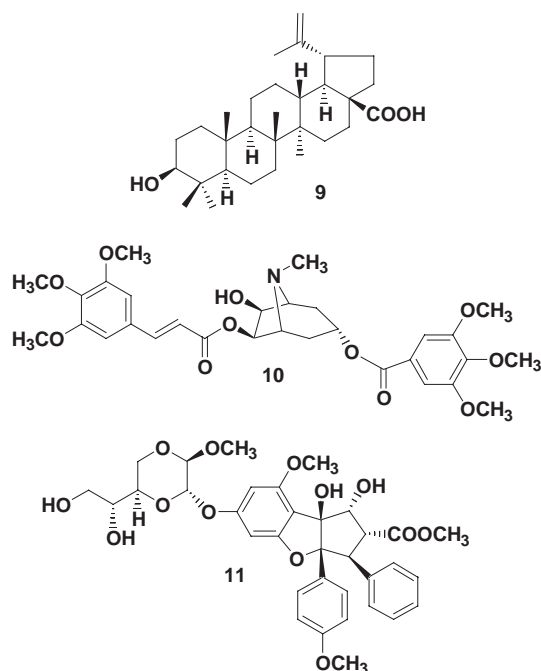

Fig. 2. NCDDG compounds from the project "Novel Strategies for Plant-derived Anticancer Agents" currently undergoing further investigation.

sion, differentiation induction, immunity enhancement, inflammation reduction, increase in apoptosis, altered gene expression, and decrease in angiogenesis) (Greenwald, 2002; Tsao et al., 2004).

Because cancer chemoprevention is designed to occur prior to the onset of cancer diagnosis, little to no toxicity can be tolerated (chemoprevention can also apply to preventing cancer recurrence in which case slightly higher toxicity levels are acceptable). As such, herbal medicines, botanicals, dietary supplements, and edible plants have all been suggested as potentially important in cancer chemoprevention due to their long history of human consumption (Park and Pezzuto, 2002; Reddy et al., 2003; Surh, 2003; Kinghorn et al., 2004). Several promising plant-derived compounds are in clinical trials through the auspices of the U.S. National Cancer Institute as potential cancer chemopreventive agents, including curcumin (Phase I colon), genistein (Phase I breast and endometrial), soy isoflavones (Phase II prostate), indole-3-carbinol (Phase I breast recurrence), perillyl alcohol (Phase I breast), various forms of retinoic acid (over 100 clinical trials in progress), phenethyl isothiocyanate (Phase I lung), green tea/epigallocatechin gallate (Phase II breast, Phase I unspecified cancer, Phase II bladder recurrence), and resveratrol (Phase I unspecified cancer) (Kelloff et al., 2000; Greenwald, 2002). These and other promising phytochemical chemopreventive agents work by various mechanisms of action targeting initiation, promotion, and progression of carcinogenesis.

Through a Program Project grant funded by the NCI entitled "Natural Inhibitors of Carcinogenesis" (P01 CA48112), we have attempted to discover promising cancer chemopreventive agents from plants (see a recent review, Kinghorn et al., 2004). This collaboration between Purdue University and UIC has involved aspects of plant procurement, extraction, in vitro and in vivo testing, isolation, structure elucidation, mechanistic studies, compound scale-up, chemistry, liquid chromatography–mass spectrometry (LC–MS), mouse mammary organ culture (MMOC), biostatistics, and structural biology.

Throughout the duration of this project, over 5000 plant accessions have been collected from over 10 countries. Emphasis has been placed on plants that are known to be edible and endemic to their country of origin, with suitable benefit-sharing agreements in place prior to plant collection. Small samples of dried plant materials are extracted using a standard solvent partitioning scheme and the ethyl acetate-soluble extracts are submitted for biological testing in a panel of in vitro bioassays. Various types of assays have been employed and updated as new information regarding appropriate chemoprevention targets is obtained, including antimutagenicity, antioxidant, HL-60 cell differentiation, quinine reductase induction, aromatase inhibition, cyclooxygenase-1 and-2 inhibition, protein kinase C inhibition, ornithine decarboxylase inhibition, and estrogen receptor antagonist/agonist bioassays (Pezzuto et al., 1999). Active extracts then undergo bioassay-guided fractionation to isolate and characterize the active compound(s), the most promising of which are tested in a MMOC assay to identify inhibitors of 7,12-dimethylbenz(a)anthracene (DMBA)-in-

duced lesions in an ex vivo setting (Mehta et al., 1997; Mehta et al., 2001). Further full-term tumorigenesis inhibition studies are performed for the most promising compounds.

As of 2003, almost 30 different types of active compounds were isolated as part of this project with over 250 total active compounds obtained (Kinghorn et al., 2004). Several of the most interesting compounds are shown in Fig. 3, including resveratrol (**12**), ixocarpalactone A (**13**), isoliquiritigenin (**14**), and four flavonoids from *Broussonetia papyrifera* Vent. (Urticaceae) (**15–18**).

Resveratrol (**12**), or 3,5,4′-trihydroxy-*trans*-stilbene, was isolated during the course of this project from *Cassia quinquangulata* Rich. (Caesalpiniaceae) collected in Peru (Jang et al., 1997). The ethyl acetate-soluble extract was found to inhibit the cyclooxygenase-1 (COX-1) enzyme (88% inhibition at 69 μg/ml) and was subjected to bioassay-guided fractionation. Resveratrol was found to inhibit COX-1 with an ED_{50} of 15 μM and had no activity on COX-2, indicating the selectivity of the compound. Resveratrol was then found to inhibit the development of DMBA-induced preneoplastic lesions in a MMOC model of carcinogenesis (Jang et al., 1997). Tested in a two-stage [using DMBA as an initiator and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) as a promoter] full-term mouse model, resveratrol was found to inhibit tumorigenesis. Further biological studies of resveratrol are ongoing (Bhat et al., 2001; Bhat and Pezzuto, 2001) and another group at the University of Michigan has begun Phase I clinical trials to determine the ability of resveratrol to prevent cancer in healthy volunteers (see http://cancer.gov/clinicaltrials/CCUM-2004-0535).

Ixocarpalactone A (**13**) was isolated during our project from the edible plant *Physalis philadelphica* Lam. (Solanaceae),
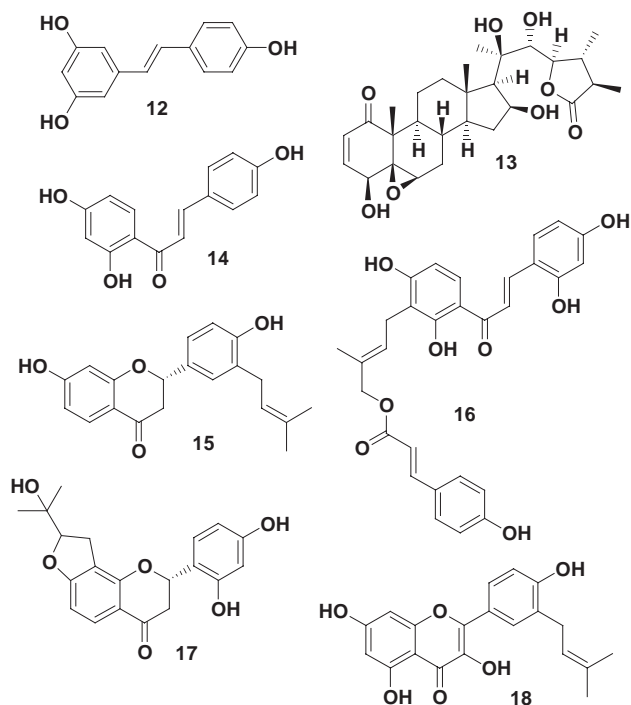

Fig. 3. Examples of highly active chemopreventive compounds from medicinal plants isolated as part of the "Natural Inhibitors of Carcinogenesis" project.

commonly known as tomatillo, grown from seed (Su et al., 2002). Tomatillos are used as an ingredient in Latin American foods such as enchiladas and salsas. An ethyl acetate-soluble extract of the leaves and stems was found to induce the quinine reductase (QR) enzyme, a Phase II enzyme responsible for metabolism of chemical carcinogens (Dinkova-Kostova and Talalay, 2000). Bioassay-guided fractionation led to the isolation of ixocarpalactone A, as well as numerous other isolates. Ixocarpalactone A induced QR with a concentration required to double activity (CD) of 0.32 μM, a concentration to inhibit 50% cell growth (IC_{50}) of 7.54 μM, and a chemopreventive index ($CI=IC_{50}/CD$) of 24. Ixocarpalactone A also inhibited the transformation of murine epidermal JB6 cells.

Isoliquiritigenin (**14**) was isolated during this project from the seeds of *Dipteryx odorata* Willd. (Fabaceae), a botanical dietary supplement commonly known as tonka bean, collected in Peru (Jang et al., 2003). The ethyl acetate-soluble extract was active in the QR bioassay and was subjected to bioassay-guided fractionation. Isoliquiritigenin was isolated as an active component with a CD value of 3.8 μM, IC_{50} of 27.3 μM, and a CI of 7.2. Isoliquiritigenin was then tested at 10 μg/ml in the MMOC ex vivo bioassay and was found to be active inhibiting induction of 76% of lesions. Isoliquiritigenin seems to be worthy of further biological testing.

Four potent aromatase inhibitors [(2S)-abyssinone II (**15**), 3′-[γ-hydroxymethyl-(E)-γ-methylallyl]-2,4,2′,4′-tetrahydroxychalcone 11′-O-coumarate (**16**), (2S)-2′,4′-dihydroxy-2′′-(1-hydroxy-1-methylethyl)dihydrofuro[2,3-h]flavanone (**17**), and isolicoflavonol (**18**)] were isolated from the edible plant *Broussonetia papyrifera* Vent. (Urticaceae), collected in Illinois (Lee et al., 2001). The ethyl acetate-soluble extract of this plant inhibited the enzyme aromatase, which is the rate-limiting enzyme in the production of estrogen. The inhibition of aromatase in post-menopausal women has been found to reduce the recurrence of breast cancer (Johnston and Dowsett, 2003; Smith and Dowsett, 2003). Compounds **15**–**18** were isolated using bioassay-guided fractionation and found to inhibit aromatase (**15**: $IC_{50}=0.4$ μM, **16**: $IC_{50}=0.5$ μM, **17**: $IC_{50}=0.1$ μM, **18**: $IC_{50}=0.1$ μM). These four compounds are currently being developed under the Rapid Access to Preventive Intervention Development (RAPID) program through NCI (see http://dcp.nci.nih.gov/cb/announcements/rapid.html).

Challenges in drug discovery from medicinal plants

Despite the evident successes of drug discovery from medicinal plants, future endeavors face many challenges. Pharmacognosists, phytochemists, and other natural product scientists will need to continuously improve the quality and quantity of compounds that enter the drug development phase to keep pace with other drug discovery efforts (Butler, 2004). The process of drug discovery has been estimated to take an average of 10 years upwards (Reichert, 2003) and cost more than 800 million dollars (Dickson and Gagnon, 2004). Much of this time and money is spent on the numerous leads that are discarded during the drug discovery process. In fact, it has been estimated that only one in 5000 lead compounds will

successfully advance through clinical trials and be approved for use. Lead identification is the first step in a lengthy drug development process (Fig. 4). Lead optimization (involving medicinal and combinatorial chemistry), lead development (including pharmacology, toxicology, pharmacokinetics, ADME [absorption, distribution, metabolism, and excretion], and drug delivery), and clinical trials all take a considerable length of time.

Drug discovery from medicinal plants has traditionally been lengthier and more complicated than other drug discovery methods. As such, many pharmaceutical companies have eliminated or scaled down their natural product research (Butler, 2004; Koehn and Carter, 2005). At NCI, contracts for the collection of plants that have been operating for nearly 20 years in the Americas, Africa, Madagascar, and Southeast Asia were recently suspended due to reallocation of NCI funds for new initiatives aimed at improving diagnosis and prevention, as well as expediting the translation of drugs from the development phase to clinical use (Dr. G.M. Cragg, National Cancer Institute, Frederick, Maryland, personal communication to A.D. Kinghorn). In addition, as academic pharmacy departments redirect their focus towards the production of clinical and community pharmacists, the emphasis on pharmaceutical research and development related to medicinal plant and natural product drug discovery in academic pharmacy departments is declining. Although the trend towards a loss of teaching and research positions in pharmacognosy has been in evidence at U.S. institutions of pharmacy education, several positive steps can be seen recently towards reversing this trend, such as the development of the National Center for Natural Products Research at the University of Mississippi, the establishment of National Institutes of Health (NIH) Botanical Centers at the University of Illinois at Chicago and the University of Arizona, and the creation of an endowed chair in natural products chemistry and pharmacognosy at The Ohio State University, currently held by one of the co-authors of this review (A.D. Kinghorn). Pharmacognosists and natural product scientists can also look for suitable employment in other
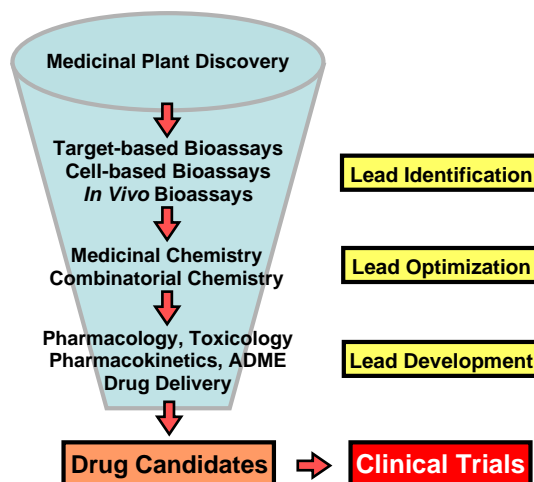

Fig. 4. Schematic of typical medicinal plant drug discovery and development (figure courtesy of Dr. Robert W. Brueggemeier, College of Pharmacy, The Ohio State University).

academic departments such as biology, chemistry, ecology, and nutrition to continue research investigations on medicinal plants.

Because drug discovery from medicinal plants has traditionally been so time-consuming, faster and better methodologies for plant collection, bioassay screening, compound isolation, and compound development must be employed (Do and Bernard, 2004; Koehn and Carter, 2005). Innovative strategies to improve the process of plant collection are needed, especially with the legal and political issues surrounding benefit-sharing agreements (Rosenthal, 2002; Soejarto et al., 2004). Redirecting plant collections from developing tropical countries to land owned by the U.S. (e.g., U.S. Virgin Islands and American Samoa) may be one such strategy.

The design, determination, and implementation of appropriate, clinically relevant, high-throughput bioassays is a difficult process for all drug discovery programs (Knowles and Gromo, 2003; Kramer and Cohen, 2004). Although the design of high-throughput screening assays can be challenging (Walters and Namchuk, 2003), after a screening assay is in place, compound and extract libraries can be tested for biological activity. Screening of extract libraries can be problematic, but new techniques, including prefractionation of extracts, can alleviate some of these issues (Butler, 2004; Koehn and Carter, 2005). Challenges in bioassay screening remain an important issue in the future of drug discovery from medicinal plants.

Improving the speed of active compound isolation will necessitate the incorporation of new technologies. Although nuclear magnetic resonance (NMR) and mass spectrometry (MS) are currently in wide use for compound identification, new methods of using NMR and MS could be applied to medicinal plant drug discovery to facilitate compound isolation (Eldridge et al., 2002; Pellecchia et al., 2002; Glish and Vachet, 2003). Also, the use of high-throughput X-ray crystallography could be applied to medicinal plant lead discovery (Blundell et al., 2002).

Compound development of drugs discovered from medicinal plants also faces unique challenges. Natural products are typically isolated in small quantities that are insufficient for lead optimization, lead development, and clinical trials. Collaborating with synthetic and medicinal chemists is necessary to determine if synthesis or semi-synthesis might be possible (Ley and Baxendale, 2002; Federsel, 2003; Lombardino and Lowe, 2004). Another technique to improve natural product compound development may involve the creation of natural product and natural-product-like libraries that combine the features of natural products with combinatorial chemistry (Hall et al., 2001a; Lee and Schneider, 2001; Eldridge et al., 2002; Feher and Schmidt, 2003; Burke et al., 2004; Ganesan, 2004; Piggott and Karuso, 2004; Tan, 2004; Koehn and Carter, 2005).

In conclusion, natural products discovered from medicinal plants (and derivatives thereof) have provided numerous clinically used medicines. Even with all the challenges facing drug discovery from medicinal plants, natural products isolated from medicinal plants can be predicted to remain an essential component in the search for new medicines.

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