Chapter 1
Drug Discovery from Plants

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Abstract Many plant-derived compounds have been used as drugs, either in their original or semi-synthetic form. Plant secondary metabolites can also serve as drug precursors, drug prototypes, and pharmacological probes. Recent developments in drug discovery from plants, including information on approved drugs and compounds now in clinical trials, are presented. There are also several plant extracts or "phytomedicines" in clinical trials for the treatment of various diseases. In the future, plant-derived compounds will still be an essential aspect of the therapeutic array of medicines available to the physician, particularly with the availability of new hyphenated analytical methods such as LC-NMR-MS and LC-SPE-NMR to accelerate their future discovery.

Keywords Natural products, Plant-derived drugs, Drug discovery, Drug development, Drug precursors, Drug prototypes, Pharmacological probes, New therapeutic agents, Clinical trials, Accelerated discovery techniques

1.1 The Role of Plants in Human History

Over the centuries humans have relied on plants for basic needs such as food, clothing, and shelter, all produced or manufactured from plant matrices (leaves, woods, fibers) and storage parts (fruits, tubers). Plants have also been utilized for additional purposes, namely as arrow and dart poisons for hunting, poisons for murder, hallucinogens used for ritualistic purposes, stimulants for endurance, and hunger suppression, as well as inebriants and medicines. The plant chemicals used for these latter purposes are largely the secondary metabolites, which are derived biosynthetically from plant primary metabolites (e.g., carbohydrates, amino acids, and lipids) and are not directly involved in the growth, development, or reproduction of plants. These secondary metabolites can be classified into several groups according to their chemical classes, such alkaloids, terpenoids, and phenolics [1].

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Arrow and dart poisons have been used by indigenous people in certain parts of the world with the principal ingredients derived from the genera *Aconitum* (Ranunculaceae), *Akocanthera* (Apocynaceae), *Antiaris* (Moraceae), *Chondrodendron* (Menispermaceae), *Strophanthus* (Apocynaceae), and *Strychnos* (Loganiaceae) [2]. Most compounds responsible for the potency of arrow and dart poisons belong to three plant chemical groups, namely the alkaloids (e.g., strychnine from *Strychnos* species), cardiac glycosides (e.g., ouabain from *Strophanthus* species), and saponins (e.g., a monodesmoside glucoside from *Clematis* species) [2].

In some cultures, toxic plant extracts were also used for murder and "trials by ordeal," where a person accused of a crime was given a noxious brew, and it was believed that if innocent, this suspect would survive this ordeal. Some of the plants well-documented for murder are henbane (*Hyoscyamus niger* L.), mandrake (*Mandragora officinarum* L.), deadly nightshade (*Atropa belladonna* L.), and some *Datura* species, all of which belong to the family Solanaceae [3]. Calabar bean (*Physostigma venenosum* Balf.) was famous for its use in trials by ordeal by people who lived on the Calabar Coast, West Africa [3]. Certain plants formerly used for arrow poisons, such as several *Aconitum* species, have also been used as medicines at lower dosages, for their analgesic and anti-inflammatory properties [4]. In fact, many compounds isolated from poisonous plants were later developed as therapeutic drugs, due to their desirable pharmacological actions [5, 6].

The use of hallucinogens in the past was usually associated with magic and ritual. However, these hallucinogens have been exploited as recreational drugs and accordingly may lead to habituation problems. Several well-recognized plants that contain hallucinogenic or psychoactive substances (the compound names are given in parentheses) include *Banisteriopsis caapi* (Spruce ex Griseb.) Morton (*N,N*-dimethyltryptamine), *Cannabis sativa* L. (Δ^9-*trans*-tetrahydrocannabinol), *Datura* species (scopolamine), *Erythroxylum coca* Lam. (cocaine), *Lophophora williamsii* (Salm-Dyck) J.M. Coult. (mescaline), *Papaver somniferum* L. (morphine), and *Salvia divinorum* Epling & Játiva (salvinorin A) [7, 8]. Several of these plants are also used as drugs due to their desired pharmacological activities, and some of the constituents of these plants have been developed into modern medicines, either in the natural form or as lead compounds subjected to optimization by synthetic organic chemistry [5, 6].

Plants have also been used in the production of stimulant beverages (e.g., tea, coffee, cocoa, and cola) and inebriants or intoxicants (e.g., wine, beer, kava) in many cultures since ancient times, and this trend continues till today. Tea (*Camellia sinensis* Kuntze) was first consumed in ancient China (the earliest reference is around CE 350), while coffee (*Coffea arabica* L.) was initially cultivated in Yemen for commercial purposes in the 9th century [3]. The Aztec nobility used to consume bitter beverages containing raw cocoa beans (*Theobroma cacao* L.), red peppers, and various herbs [3]. Nowadays, tea, coffee, and cocoa are important commodities and their consumption has spread worldwide. The active components of these stimulants are methylated xanthine derivatives, namely caffeine, theophylline, and theobromine, which are the main constituents of coffee, tea, and cocoa, respectively [9].

The most popular inebriants in society today are wine, beer, and liquor made from the fermentation of fruits and cereals. Wine was first fermented about 6000–8000 years ago in the Middle East, while the first beer was brewed around 5000–6000 BCE by the Babylonians [3]. The intoxicating ingredient of these drinks is ethanol, a by-product of bacterial fermentation, rather than secondary plant metabolites. Recent studies have shown that a low to moderate consumption of red wine is associated with reduction of mortality due to cardiovascular disease and cancer [10]. This health benefit has been suggested to be due to the presence of resveratrol, a hydroxylated stilbenoid found in the skin of grapes [11]. Kava, a beverage made from the root of *Piper methysticum* Roxb., has been a popular intoxicating beverage in Polynesia for centuries [3]. Kava is not normally consumed in this manner in the Western world, but has gained popularity as a botanical dietary supplement to ease the symptoms of stress, anxiety, and depression [12]. A study has shown that the anxiolytic activity of kava extract may be mediated in part by the kavalactone, dihydrokavain [13]. The consumption of kava has been associated with liver toxicity, although this is somewhat controversial. Recently, a study has shown that the alkaloid pipermethystine, found mostly in the leaves and stems of *Piper methysticum*, may be responsible for this toxicity [14].

Plants have formed the basis of sophisticated traditional medicine (TM) practices that have been used for thousands of years by people in China, India, and many other countries [9]. Some of the earliest records of the usage of plants as drugs are found in the Artharvaveda, which is the basis for Ayurvedic medicine in India (dating back to 2000 BCE), the clay tablets in Mesopotamia (1700 BCE), and the Eber Papyrus in Egypt (1550 BCE) [9]. Other famous literature sources on medicinal plant include "De Materia Medica," written by Dioscorides between CE 60 and 78, and "Pen Ts'ao Ching Classic of Materia Medica" (written around 200 CE) [9].

Before the realization that pharmacologically active compounds present in medicinal plants are responsible for their efficacy, the "doctrine of signatures" was often used to identify plants for treating diseases. For example, goldenrod with a yellow hue was used to cure jaundice, red-colored herbs were used to treat blood diseases, liverworts were used for liver diseases, pileworts for hemorrhoids, and toothworts for toothache [9]. In 1805, morphine became the first pharmacologically active compound to be isolated in pure form from a plant, although its structure was not elucidated until 1923 [9]. The 19th century marked the isolation of numerous alkaloids from plants (species in parentheses) used as drugs, namely, atropine (*Atropa belladonna*), caffeine (*Coffea arabica*), cocaine (*Erythroxylum coca*), ephedrine (*Ephedra* species), morphine and codeine (*Papaver somniferum*), pilocarpine (*Pilocarpus jaborandi* Holmes), physostigmine (*Physostigma venenosum*), quinine (*Cinchona cordifolia* Mutis ex Humb.), salicin (*Salix* species), theobromine (*Theobroma cacao*), theophylline (*Camellia sinensis*), and (+)-tubocurarine (*Chondodendron tomentosum* Ruiz & Pav.) [9]. Following these discoveries, bioactive secondary metabolites from plants were later utilized more widely as medicines, both in their original and modified forms [5, 6].

The correlation between the ethnomedical usage of medicinal plants and modern medicines discovered from those plants has been studied by Fabricant and Farnsworth [15]. Based on their analysis, 88 single chemical entities isolated from 72 medicinal plants have been introduced into modern therapy, many of which have the same or a similar therapeutic purpose as their original ethnomedical use [15]. Some of these plant-derived compounds, such as atropine (anticholinergic), codeine (cough suppressant), colchicine (antigout), ephedrine (bronchodilator), morphine (analgesic), pilocarpine (parasympathomimetic), and physostigmine (cholinesterase inhibitor) are still being used widely as single-agent or combination formulations in prescription drugs [5].

Nowadays, plants are still important sources of medicines, especially in developing countries that still use plant-based TM for their healthcare. In 1985, it was estimated in the Bulletin of the World Health Organization (WHO) that around 80% of the world's population relied on medicinal plants as their primary healthcare source [16]. Even though a more recent figure is not available, the WHO has estimated that up to 80% of the population in Africa and the majority of the populations in Asia and Latin America still use TM for their primary healthcare needs [17]. In industrialized countries, plant-based traditional medicines or phytotherapeuticals are often termed complementary or alternative medicine (CAM), and their use has increased steadily over the last 10 years. In the USA alone, the total estimated "herbal" sales for 2005 was $4.4 billion, a significant increase from $2.5 billion in 1995 [18]. However, such "botanical dietary supplements" are regulated as foods rather than drugs by the United States Food and Drug Administration (US FDA).

1.2 The Role of Plant-Derived Compounds in Drug Development

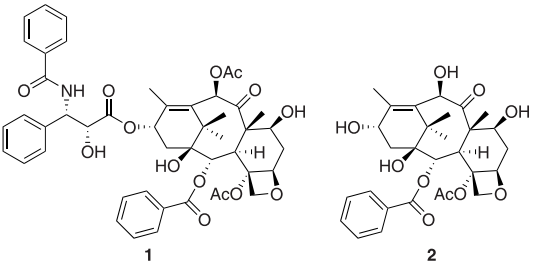
Despite the recent interest in drug discovery by molecular modeling, combinatorial chemistry, and other synthetic chemistry methods, natural-product-derived compounds are still proving to be an invaluable source of medicines for humans. The importance of plants in modern medicine has been discussed in recent reviews and reports [19–22]. Other than the direct usage of plant secondary metabolites in their original forms as drugs, these compounds can also be used as drug precursors, templates for synthetic modification, and pharmacological probes, all of which will be discussed briefly in turn in this section.

1.2.1 Plant Secondary Metabolites as Drug Precursors

Some natural products obtained from plants can be used as small-molecule drug precursors, which can be converted into the compound of interest by chemical

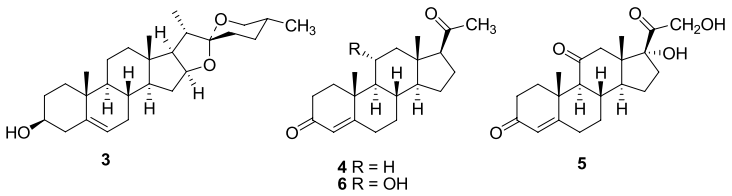
modification or fermentation methods. The semisynthetic approach is usually used to resolve the shortage of supply due to the low yield of compounds from plants and/or the high cost of total synthesis. For compounds with complex structures and many chiral centers, protracted methods may be needed for their synthesis, and thus these methods would not be feasible economically. The following examples indicate that some secondary metabolites from plants are useful drug precursors, although they are not necessarily pharmacologically active in their original naturally occurring forms.

Cropping of the bark of the slow-growing Pacific yew tree, *Taxus brevifolia* Nutt., is not a feasible method to provide sufficient amounts of the antitumor drug paclitaxel (1, Taxol) to meet the market demand (paclitaxel was originally isolated in only 0.014% w/w yield from the bark of *Taxus brevifolia*) [23]. Even though this compound can be produced by total synthesis, this has proven to be inefficient in affording large quantities of paclitaxel [24, 25]. Fortunately, 10-deacetylbaccatin III (**2**) can be isolated in relatively large amounts from the needles of other related yew species, such as *Taxus baccata* L. (a renewable resource), and can be converted chemically in several steps into paclitaxel [26, 27]. During the period 1993–2002, the main pharmaceutical manufacturer, Bristol-Myers Squibb, adopted the semisynthetic method developed by the Holton research group to produce paclitaxel from 10-deacetylbaccatin III [27, 28]. Since 2002, Bristol-Myers Squibb has produced paclitaxel using a plant cell culture method, which will be mentioned in section 1.4 of this chapter [29].

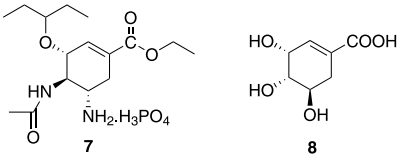


Diosgenin (**3**), a steroidal sapogenin obtained from the tubers of various *Dioscorea* species that grow in Mexico and Central America, can be converted chemically in several steps into progesterone (**4**), a hormone that can be used as a female oral contraceptive [30]. Originally, progesterone was isolated from sow ovaries with a very low yield (20 mg from 625 kg of ovaries), and later was synthesized from cholesterol with very low efficiency [31]. Progesterone is also a key intermediate for the production of cortisone (**5**), an important anti-inflammatory drug. Progesterone can be converted into 11α-hydroxyprogesterone (**6**) by microbial hydroxylation at C-11, followed by chemical reactions, to produce

cortisone (**5**) [32, 33]. Until now, diosgenin (**3**) is still an important starting material for the production of various steroid hormones.



Oseltamivir phosphate (**7**, Tamiflu) is an orally active neuraminidase inhibitor developed for the treatment and prophylaxis of influenza viruses A and B [34, 35]. The starting material for the oseltamivir synthesis is (−)-shikimic acid (**8**), an important biochemical intermediate in plants and microorganisms [36]. Previously, shikimic acid was extracted solely from the fruits of the shikimi tree (*Illicium verum* Hook.f.), also known as star anise, which contains a large amount of this compound [37]. Later on, shikimic acid was obtained from the fermentation of genetically engineered *Escherichia coli* strains, which are deficient in the shikimate kinase gene [38]. Currently, Roche, the drug manufacturer, still relies on both extraction and fermentation methods to obtain ton quantities of shikimic acid [37]. Several routes for the production of oseltamivir independent of shikimic acid have been developed [36, 39], but these alternatives are still not cost efficient [37].

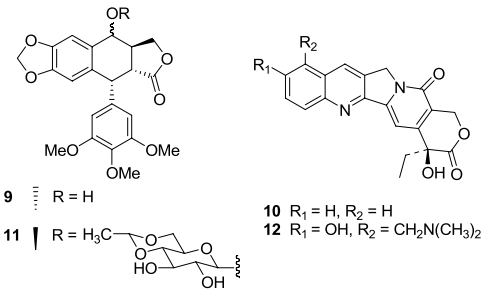


1.2.2 Plant Secondary Metabolites as Drug Prototypes

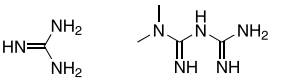
Sneader has defined a drug prototype as "the first compound discovered in a series of chemically related therapeutic agents" [5]. As of 1996, from a total of 244 drug prototypes identified in one analysis from minerals, plants, animals, microbes, and chemical sources, plant secondary metabolites contributed 56 of these (23%) [5]. With advances in organic chemistry, medicinal chemists started preparing analogs from these drug prototypes to provide safer and more potent drugs. Sometimes, new compounds with novel pharmacological properties have

been developed in the process of developing such derivatives. In the following examples, podophyllotoxin, camptothecin, and guanidine have been selected as drug prototypes with analogs having the same pharmacological action as the parent compound, while atropine is a drug prototype that has furnished many analogs that have additional pharmacological properties.

Several antineoplastic compounds isolated from plants, such as podophyllotoxin (**9**) and camptothecin (**10**), are too toxic or not water soluble enough for clinical application, and analogs with higher therapeutic indices such as etoposide (**11**, Vepesid) and topotecan (**12**, Hycamtin) have been developed in consequence [40, 41]. Due to their unique modes of anticancer activities, there is much interest in the clinical development of further derivatives of paclitaxel (**1**) and camptothecin (**10**) as anticancer therapeutic drugs [28, 41–43]. According to a recent review, of the 2255 cancer clinical trials recorded as of August 2003, 310 (or 13.7%) and 120 (or 5.4%) of the trials involved taxane- and camptothecin-derived drugs, respectively [43]. In 2002, it was estimated that the combined sales of paclitaxel and docetaxel (both taxanes), and topotecan and irinotecan (both based on the parent molecule camptothecin) constituted over 30% of the total global sales of cytotoxic drugs [44].



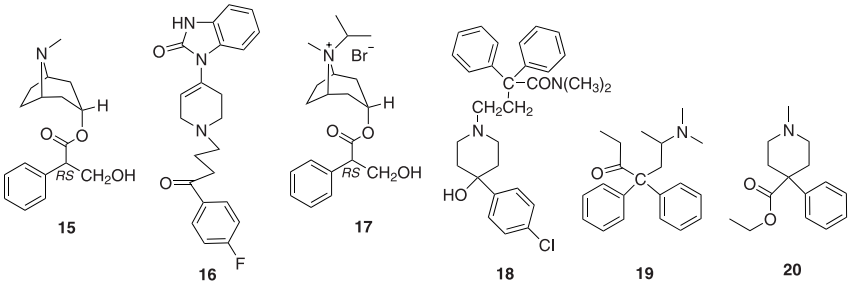
Guanidine (**13**) is a natural product with good hypoglycemic activity isolated from *Galega officinalis* L., but is too toxic for clinical use [45]. Many derivatives of guanidine have been synthesized, and metformin (dimethylbiguanide) (**14**) was later found to be clinically suitable for treatment of type II diabetes [46].



13 **14**

Atropine (**15**) is an artifact of the tropane alkaloid (−)-hyoscyamine, which racemizes during the extraction process from its plant of origin (*Atropa belladonna*). Atropine is a competitive antagonist of muscarinic acetylcholine

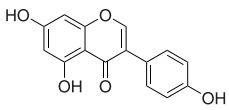
receptors (antimuscarinic agent). Atropine is sometimes used in the ophthalmology area as a mydriatic agent, and has additional therapeutic uses as an antispasmodic. It is also used as a premedication for anesthesia, to decrease bronchial and salivary secretions, and to block the bradycardia (low heart rate) associated with the administration of anesthetic drugs [5]. Biological and physiological studies of a large number of synthetic atropine analogs have led to the introduction of new drugs with different therapeutic applications than the parent compound. Examples of drugs derived from the basic atropine skeleton include droperidol (**16**, antipsychotic), ipratropium bromide (**17**, bronchodilator for the treatment of asthma), loperamide (**18**, antidiarrheal), methadone (**19**, a morphine substitute for addicts), and pethidine (**20**, analgesic) [5].



1.2.3 Plant Secondary Metabolites as Pharmacological Probes

In addition to their direct contribution as drugs or drug protopyes to cure human disease, secondary metabolites of plant origin, such as phorbol esters and genistein, can be used as "pharmacological probes." Pharmacological probes help researchers to understand the mechanism of action of intracellular signal transductions and biological mechanisms related to human disease, which can aid the design of better drugs.

Genistein (**21**), an isoflavone found naturally in soybean (*Glycine max* Merr.), is an inhibitor of various protein tyrosine kinases (PTK), which are essential enzymes involved in intracellular signal transduction [47]. Genistein has been used to probe the interaction between PTK and cyclic nucleotide-gated (CNG) channels, which are important in mammalian olfactory and visual systems [48, 49]. By observing the effect of genistein on the CNG channels containing either homomeric or heteromeric subunits, specific subunits containing binding sites for PTKs can be identified [48]. Furthermore, the mechanism of inhibition of the CNG channels by PTKs has been studied with the aid of genistein as a probe [49].



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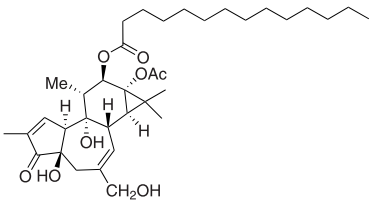
Phorbol is a tetracyclic diterpenoid plant secondary metabolite isolated as a hydrolysis product of croton oil from the seeds of *Croton tiglium* L. [50]. Various 12,13-diesters of phorbol have the capacity to act as tumor promoters, due in part to their role as protein kinase C (PKC) activators [51–53]. The most abundant phorbol ester derivative of croton oil, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (**22**), has been used in biomedical research in standard laboratory models of carcinogenesis promotion [54–56].



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1.3 Recent Developments in Drug Discovery from Plants

Despite the large number of drugs derived from total synthesis, plant-derived natural products still contribute to the overall total number of new chemical entities (NCE) that continue to be launched to the market. Several reviews on drug discovery and development from natural sources (plants, marine fauna, microbes) have been published recently [42, 57–59]. The following sections will cover specifically the plant-derived drugs newly launched since 2001 and examples of some plant-derived compounds currently in clinical trials.

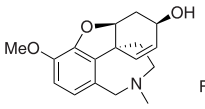
1.3.1 New Plant-Derived Drugs Launched Since 2001

In the past 6 years, five new drugs derived from natural products, namely, apomorphine hydrochloride, galanthamine hydrobromide, nitisinone, tiotropium bromide, and varenicline, have been approved by the US FDA. The following is a brief description of each drug and their therapeutic use.

Galantamine (**23**, Razadyne, Reminyl, Nivalin) was first marketed in 2001 in the USA for the symptomatic treatment of patients with early-onset Alzheimer's

disease [58]. Galantamine (also known as galanthamine) is an alkaloid that was initially isolated from the snowdrop (*Galanthus woronowii* Losinsk.) in the early 1950s, and has since been found in other plants in the family Amaryllidaceae [60]. Galantamine slows the process of neurological degeneration by inhibiting acetylcholinesterase as well as binding to and modulating the nicotinic acetylcholine receptor [60, 61]. Due to the limited availability of the plants of origin of this compound, galantamine is now produced by total synthesis.

Nitisinone (**24**, Orfadin) was approved by the FDA in 2002 for the treatment of hereditary tyrosinemia type 1 (HT-1) [58]. HT-1 is a rare pediatric disease caused by a deficiency of fumaryl acetoacetate hydrolase (FAH), an enzyme essential in the tyrosine catabolism pathway. FAH deficiency leads to the accumulation of toxic substances in the body, resulting in liver and kidney damage [62]. Nitisinone is a derivative of leptospermone (**25**), a new class of herbicide from the bottlebrush plant [*Callistemon citrinus* (Curtis) Skeels]. Both nitisinone and leptospermone inhibit 4-hydroxyphenyl pyruvate dioxygenase (HPPD), the enzyme involved in plastoquinone and tocopherol biosynthesis in plants [63]. In humans, inhibition of HPPD prevents tyrosine catabolism, leading to the accumulation of tyrosine metabolites, 4-hydroxyphenyl pyruvic acid and 4-hydroxyphenyl lactic acid, which can be excreted through the urine [64].



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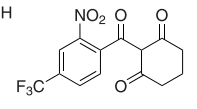

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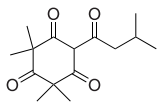

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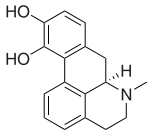
Apomorphine (**26**, Apokyn) was approved by the FDA in 2004 as an injectable drug for the symptomatic treatment for Parkinson's disease patients during episodes of "hypomobility" (e.g., persons unable to move or to perform daily activities) [65]. Apomorphine is a synthetic derivative of morphine (**27**), but unlike morphine, apomorphine does not have opioid analgesic properties, and instead is a short-acting dopamine D_1 and D_2 receptor agonist [66].



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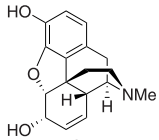

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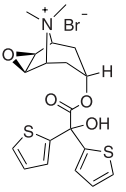
Tiotropium bromide (**28**, Spiriva), an atropine analog, was approved by the FDA in 2005 for the treatment of bronchospasm associated with chronic obstructive pulmonary disease (COPD), including chronic bronchitis and emphysema [67].



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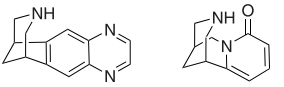
Varenicline (**29**, Chantix), based on the plant quinolizidine alkaloid, cytisine (**30**), has been approved by the FDA since 2006 as an aid to smoking cessation [68–70]. Cytisine (**30**), an alkaloid isolated from *Cytisus laburnum* L., has been used to treat tobacco dependence in Eastern Europe (Bulgaria, Germany, Poland, and Russia) for the last 40 years [71]. Cigarette smoking has been linked to several diseases including cardiovascular disease, COPD, many cancers (particularly lung, mouth, and esophageal), and pregnancy-related complications. Varenicline (**29**) is a partial agonist with a high affinity for the $\alpha_4\beta_2$ nicotinic acetylcholine receptor, and is a full agonist at α_7 neuronal nicotinic receptors [70].



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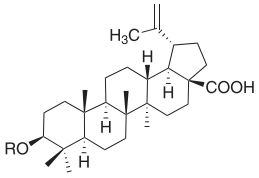
1.3.2 Examples of Plant-Derived Compounds Currently Involved in Clinical Trials

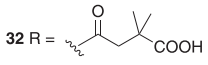
Although relatively few plant-derived drugs have been launched onto the market the last 6 years, many plant-derived compounds are currently undergoing clinical trial for the potential treatment of various diseases. The majority of such drugs under clinical development are in the oncological area, including new analogs of known anticancer drugs based on the camptothecin-, taxane-, podophyllotoxin-, or vinblastine-type skeletons [42]. Examples of compounds with carbon skeletons different from the existing plant-derived drugs used in cancer chemotherapy will be discussed below, namely, betulinic acid, ceflatonine (homoharringtonine), combretastatin A4 phosphate, ingenol-3-angelate, phenoxodiol, and protopanaxadiol. In the antiviral area, bevirimat and celgosivir are currently undergoing clinical trials for the treatment of human immunodeficiency viral (HIV) and hepatitis C viral (HCV) infections, respectively. Capsaicin is in clinical trial for the treatment of severe postoperative pain, while huperzine is being developed for the treatment of Alzheimer's disease.

Betulinic acid (**31**) is a lupane-type triterpene that is widely distributed in the plant kingdom, and this compound, along with various derivatives, has been shown to have anticancer, antibacterial, antimalarial, anti-HIV, anthelmintic, anti-inflammatory, and antioxidant properties [72, 73]. In 1995, a research group from the University of Illinois at Chicago reported that betulinic acid selectively inhibited human melanoma in both *in vitro* and *in vivo* model systems, and induced apoptosis in Mel-2 human melanoma cells [74]. This compound was further developed under the Rapid Access to Intervention Development program of the United States National Cancer Institute [75], and is currently undergoing phase I/II clinical trials for treatment of dysplastic melanocytic nevi, a preliminary symptom that may lead to melanomas of the skin [76].

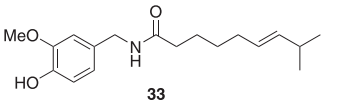
Bevirimat (**32**, PA-457), a semisynthetic compound derived from betulinic acid, is being developed by Panacos Pharmaceuticals (Watertown, MA, USA) as a new class of antiretroviral drug [77]. Bevirimat blocks HIV-1 maturation by disrupting a late step in the *Gag* processing pathway, causing the virions released to be noninfectious, thus terminating the viral replication [78]. This compound is currently undergoing phase II clinical trials, and phase III trials are expected to commence in 2007 [77, 79].



31 R = H

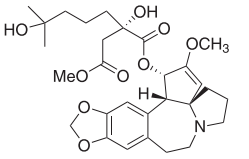


Capsaicin (**33**) is a capsaicinoid-type amide that causes the burning sensation in the mouth associated with eating chilli peppers [80]. Upon topical application, capsaicin desensitizes the neurons and lowers the threshold for thermal, chemical, and mechanical nociception by direct activation of the transient receptor potential channel, vanilloid subfamily member 1 [80, 81]. Low-concentration capsaicin (0.025–0.075%) creams and dermal patches are now available without prescription to relieve the pain associated with osteoarthritis, rheumatoid arthritis, postherpetic neuralgia, psoriasis, and diabetic neuropathy [82]. Anesiva (San Fransisco, CA, USA) has developed a capsaicin formulation for internal use, for the treatment of severe postoperative pain, post-traumatic neuropathic pain, and musculoskeletal diseases, which is currently undergoing various phase II clinical trials [83].



33

Ceflatonine (**34**), a synthetic version of homoharringtonine produced by ChemGenex Pharmaceuticals (Menlo Park, CA, USA), is currently undergoing phase II/III clinical trials for the treatment of patients with chronic myeloid leukemia that is resistant to the first-line therapy, Gleevec [84]. Homoharringtonine is an alkaloid isolated from the Chinese evergreen tree *Cephalotaxus harringtonia* K. Koch. [84]. Homoharringtonine affects several cellular pathways, including the regulation of genes associated with apoptosis and angiogenesis [85].



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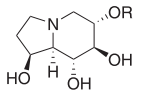
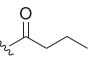
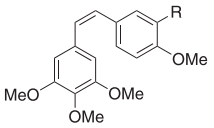
Celgosivir (**35**, MX-3253), developed by MIGENIX (Vancouver, Canada), is a semisynthetic derivative of the alkaloid castanospermine (**36**), which is isolated from the Australian tree *Castanospermum australe* A. Cunningham ex R. Mudie [86, 87]. Celgosivir is an α-glucosidase I inhibitor and has shown in vitro synergy with various interferons [88]. Celgosivir is currently undergoing phase IIb clinical trials as a combination therapy with peginterferon α2b and ribavirin for the treatment of patients with chronic HCV infection [89].



35 R = 

36 R = H

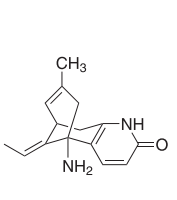
Combretastatin A4 phosphate (**37**, CA4P) is a disodium phosphate prodrug of the natural stilbene combretastatin A4 (**38**) isolated from the South African tree *Combretum caffrum* Kuntze [90]. CA4P is being developed by OXiGENE (Waltham, MA, USA) to treat anaplastic thyroid cancer in combination with other anticancer drugs and also for myopic macular degeneration, both in phase II clinical trials [91]. Combretastatin is a vascular targeting agent that functions by destroying existing tumor vasculature by inducing morphological changes within the endothelial cells [90].



37 R = OPO_3Na_2

38 R = OH

Huperzine A (**39**) is an alkaloid with a potent acetylcholinesterase inhibitory activity isolated from the Chinese club moss *Huperzia serrata* (Thunb. ex Murray) Trevis. [92]. Huperzine A is currently available in the USA as "nutraceutical" or "functional food". The National Institute on Aging, at the National Institutes of Health (Bethesda, USA) [93], in collaboration with Neuro-Hitech (New York, NY, USA) [94] are currently evaluating the safety and efficacy of huperzine A in a phase II clinical trial [95]. A prodrug of huperzine A, ZT-1 (**40**), is currently being evaluated by Debiopharm (Lausanne, Switzerland) in phase II clinical trials for the potential treatment of Alzheimer's disease, and has shown efficacy in patients with mild to moderate symptoms [96].



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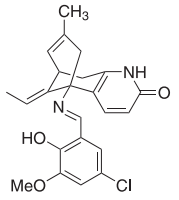


40

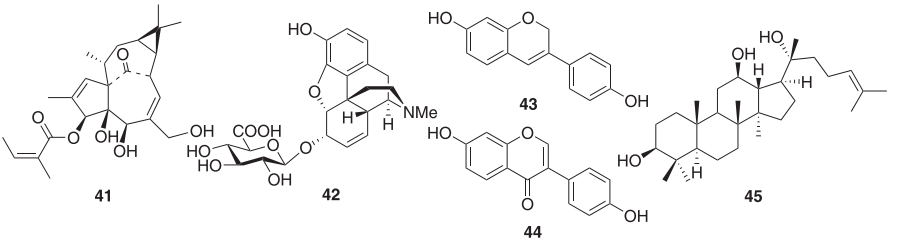
Ingenol 3-angelate (**41**, PEP005) is a diterpene ester isolated from the medicinal plant *Euphorbia peplus* L., a species used traditionally to treat skin conditions such as warts and actinic keratoses [97]. PEP005 kills tumor cells via two mechanisms: (1) by inducing primary necrosis of tumor cells, and (2) by potently activating PKC. This is also associated with an acute T-cell-independent inflammatory response that is characterized by a pronounced neutrophil infiltration [98]. PEP005, developed by Peplin (Brisbane, Australia), is currently undergoing phase II clinical trials as a topical formulation for the treatment of actinic keratosis and basal cell carcinoma [99].

Morphine (**27**), an opiate analgesic alkaloid isolated from *Papaver somniferum*, is a drug that is still used widely today for the alleviation of severe pain [5]. Morphine is metabolized into morphine-3-glucuronide and morphine-

6-glucuronide (**42**, M6G) in the human body; but of these two metabolites, only M6G possesses analgesic activity [100, 101]. M6G is being developed by CeNeS (Cambridge, UK) as a treatment for postoperative pain, and is currently undergoing phase III trials in Europe, with phase III clinical trials in the USA expected to commence in 2007 [102]. The results of clinical testing to date have shown that M6G gives the same postoperative pain relief as morphine, but causes less postoperative nausea and vomiting [102].

Phenoxodiol (**43**), a synthetic analog of daidzein (**44**), an isoflavone from soybean (*Glycine max* Merr.), is being developed by Marshall Edwards (North Ryde, Australia) for the treatment of cervical, ovarian, prostate, renal, and vaginal cancers [103]. Phenoxodiol is a broad-spectrum anticancer drug that induces cancer cell death through inhibition of antiapoptotic proteins including XIAP and FLIP [104]. Phase III clinical trials of phenoxodiol as a treatment for ovarian cancer has started in Australia, with phase II trials currently underway in the USA [105].

Protopanaxadiol (**45**), a triterpene aglycone hydrolyzed from various Korean ginseng (*Panax ginseng* C. A. Mey.) saponins [106], has been shown to exhibit apoptotic effects on cancer cells through various signaling pathways, and has also been reported to be cytotoxic against multidrug-resistant tumors [106–108]. PanaGin Pharmaceuticals (British Columbia, Canada) is developing protopanaxadiol (Pandimex) for the treatment of lung cancer and other solid tumors, and is currently undergoing phase I clinical study in the USA [109]. Pandimex has been marketed in the People's Republic of China under conditional approval for the treatment of advanced cancers of the lung, breast, pancreas, stomach, colon, and rectum [110].

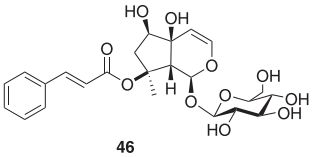


1.3.3 Plant Extracts Currently Involved in Clinical Trials

There are new forms of registered plant-derived medicines (phytomedicines) that are not single chemical entities. These more complex drugs are subjected to quality control via extract standardization procedures involving either or both compounds with known biological activity or inactive "marker compounds"

present in high concentration [111]. The following are examples of standardized plant extracts that have undergone clinical trial for the treatment of several diseases, including osteoarthritis and cancer, and as a pain reliever.

Devil's Claw (*Harpagophytum procumbens* DC.) has been used for thousands of years in Africa for the treatment of fever, rheumatoid arthritis, and skin conditions, and is currently available as an alternative treatment for pain and osteoarthritis [112]. Harpagoside (**46**), one of the major components of the plant, has been shown to suppress lipopolysaccharide-induced inducible nitric oxide synthase and cyclooxygenase (COX)-2 expression through inhibition of nuclear factor-κB activation [113]. Several clinical trials have shown a *Harpagophytum procumbens* extract containing 50–60 mg of harpagoside to be effective in treating pain [114]. This *Harpagophytum procumbens* extract is currently undergoing phase II clinical trials in the USA for the treatment of hip and knee osteoarthritis [115].



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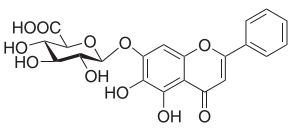
Flavocoxid (Limbrel), a proprietary blend of natural flavonoids from *Scutellaria baicalensis* Georgi and *Acacia catechu* Willd., is being marketed in the USA by Primus Pharmaceuticals (Scottsdale, AZ, USA) under prescription as a "medical food" therapy for osteoarthritis [116]. A medical food is not a drug, nor a dietary supplement, and is defined by the FDA as a "formulated food that is consumed under the supervision of a physician and is intended for the specific management of a disease" [117]. Flavocoxid is currently undergoing a phase I clinical trial in the USA for the treatment of knee osteoarthritis. The active components of flavocoxid include baicalin (**47**) and cathechin (**48**), two flavonoids with anti-inflammatory and antioxidant properties [118]. This product works by inhibiting the cyclooxygenase (COX-1 and COX-2) and lipoxygenase (5-LOX) enzyme systems, two major inflammatory pathways involved in osteoarthritis that process arachidonic acid into inflammatory metabolites [119].



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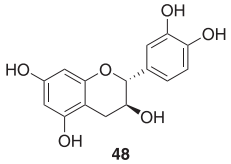

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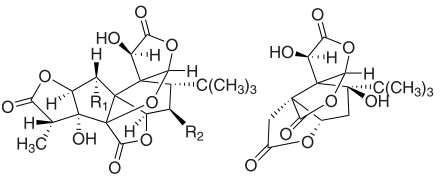
Ginkgo extracts are produced from the dried leaves of the *Ginkgo biloba* L. tree, a unique species with no close living relatives, that has been described as a "living fossil." The standardized ginkgo extract (EGb 761) contains approximately 24% flavone glycosides (primarily quercetin, kaempferol, and isorhamnetin) and 6% terpenoid lactones [2.8–3.4% ginkgolides A (**49**), B (**50**), and C (**51**), and 2.6–3.2% bilobalide (**52**)] [120]. Ginkgo extract is used for the treatment of early-stage Alzheimer's disease (AD), vascular dementia, peripheral claudication, and tinnitus of vascular origin [121]. Several reviews on the studies of ginkgo extract for the treatment of patients with Alzheimer's disease and dementia have been published [122–124]. In the USA, *Ginkgo biloba* extract (240 mg/day) is currently undergoing phase III clinical trials to prevent dementia and the onset of Alzheimer's disease in older individuals [125].

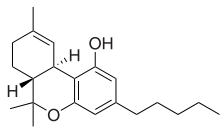


49 R_1 = H, R_2 = H
50 R_1 = OH, R_2 = H
51 R_1 = OH, R_2 = OH

52

Mistletoe (*Viscum album* L.) extract (Iscador) has been used as a complementary treatment in cancer patients in various European countries (e.g., Austria, Germany, Switzerland, and the United Kingdom) [126]. In the USA, mistletoe extract is currently undergoing phase II clinical trials as a supplemental treatment for lung cancer patients receiving conventional chemotherapy [127], and in phase I trials as a combination drug with gemcitabine (a synthetic antitumor drug) for patients with advanced solid tumors [128]. Mistletoe extract has been shown to have cytotoxicity against tumor cells and immunomodulatory activity, but the mechanism of action is poorly understood [126]. Mistletoe contains a cytotoxic lectin (viscumin) and several cytotoxic proteins and polypeptides (viscotoxins) that have been shown to induce tumor necrosis, increase natural killer cell activity, increase the production of interleukins 1 and 6, activate macrophages, induce programmed cell death (apoptosis), and protect DNA in normal cells during chemotherapy [129–132].

Sativex, developed by GW Pharmaceuticals (Wiltshire, UK), is a standardized extractive of *Cannabis sativa* L. with an almost 1:1 ratio of the cannabinoids, Δ^9-tetrahydrocannabinol (**53**) and cannabidiol (**54**), for the treatment of neuropathic pain in patients with multiple sclerosis [133]. In 2005, Sativex oromucosal spray was approved by Health Canada as an adjunctive treatment for the symptomatic relief of neuropathic pain in multiple sclerosis patients [134]. In the USA, Sativex began phase III clinical trials for multiple sclerosis patients in 2006 [135].



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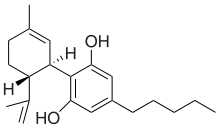

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1.4 Recent Trends and Future Directions

Plant-derived and other natural product secondary metabolites have provided many novel prototype bioactive molecules, some of which have led to important drugs that are available on the market today. In spite of this, in the last 10 years or so, most large pharmaceutical companies have either terminated or scaled down their natural products drug-discovery programs, largely in favor of performing combinatorial chemistry, which can generate libraries consisting of millions of compounds [58, 136]. The roles of large pharmaceutical companies in the field of natural products have now been taken over to some extent by small biotechnology companies, which are specializing in lead identification from natural product extracts and the development of these leads into drugs [58, 137]. Many of the plant-derived drugs currently undergoing clinical trials were obtained and promoted by these emerging "biotech" companies, some of which were mentioned in the previous section.

In the past, drug discovery of bioactive compounds from plants was time-consuming, and the process of identifying the structures of active compounds from an extract could take weeks, months, or even years, depending on the complexity of the problem. Nowadays, the speed of bioassay-guided fractionation has been improved significantly by improvements in instrumentation such as high-performance liquid chromatography (HPLC) coupled to mass spectrometry (MS)/MS (liquid chromatography, LC-MS), higher magnetic field-strength nuclear magnetic resonance (NMR) instruments, and robotics to automate high-throughput bioassays. The introduction of capillary NMR (cap-NMR) spectroscopy is a recent major breakthrough for the characterization of compounds that are extremely limited in quantity in their organisms of origin [138, 139].

The high sensitivity of the cap-NMR probe has allowed for the combination of NMR spectroscopy with other analytical "hyphenated" techniques, such as LC-NMR-MS and LC-solid phase extraction (SPE)-NMR [140, 141]. The LC-NMR-MS technique generally requires deuterated solvents during the chromatographic separation, or alternatively, solvent suppression can be used for nondeuterated solvents [141, 142]. In contrast, the LC-SPE-NMR technique does not require deuterated solvents during the chromatographic separation, and, furthermore, it allows for sample enrichment through repeated chromatographic runs using SPE before NMR analysis [140]. A state-of-the-art integrated system for LC-NMR-MS and LC-SPE-NMR-MS has been developed and the hardware can be switched from LC-NMR-MS to LC-SPE-NMR-

MS with minimal reconfiguration [140]. LC-SPE-NMR in combination with HPLC-electrospray ionization mass spectrometry (ESIMS) has been used for the rapid identification of compounds present in crude extracts of plants, as exemplified by the identification of sesquiterpene lactones and esterified phenylpropanoids in *Thapsia garganica* L. [143], and the characterization of constituents of *Harpagophytum procumbens* [144].

The development of automated high-throughput techniques has allowed for rapid screening of plant extracts; thus, the biological assay is no longer the rate-limiting step in the drug-discovery process. With advances in data handling systems and robotics, 100,000 samples can be assayed in just over 1 week when using a 384-well format [42]. Screening of plant extract libraries can be problematic due to the presence of compounds that may either autofluoresce or have UV absorptions that interfere with the screen readout, but prefractionation of extracts can be used to alleviate some of these types of problems. Also, most high-throughput screening assay methods have been developed with computational filtering methods to identify and remove potentially problematic compounds that can give false-positive results [145].

In the future, the routine use of NMR "hyphenated" techniques will allow for quick "dereplication" (a process of eliminating known and active compounds in the plant extracts that have been studied previously), and high-throughput screening will permit the rapid identification of the active compounds [140]. For example, duplicate SPE plates can be generated during the HPLC separation, with one plate used to prepare samples for high-throughput screening, while the other plate is kept as a reference. The structure(s) of compounds in wells of these plates that show(s) activity can be determined by cap-NMR and MS, and known compounds can be ruled out quickly based on their NMR spectroscopic and MS information. In instances where the active compound has a new structure, further isolation can be carried out from the plant material, provided there is enough sample. Alternatively, the compound can be synthesized for further bioassay, and combinatorial chemistry can be used to design new analogs based on the parent molecules.

Adequate and continuous supplies of plant-derived drugs are essential to meet the market demand. For compounds that are uneconomical to synthesize, and only available in a small quantities from plants, the use of plant cell cultures is an alternative production method. Plants accumulate secondary metabolites at specific developmental stages, and by manipulating the environmental conditions and medium, many natural products have been synthesized in cell cultures in larger percentage yields than those evident in whole plants [146, 147]. Paclitaxel (**1**) has been produced successfully by plant cell fermentation (PCF) technology, and, as mentioned earlier, the supply of the important anticancer drug, paclitaxel, by its manufacturer, Bristol-Myers Squibb, is now obtained by PCF technology [148–150]. Other plant-derived compounds that can currently be produced by cell cultures include the *Catharanthus* alkaloids [151], diosgenin from *Dioscorea* [152], and the *Panax ginseng* ginsenosides [153].

In conclusion, plants have provided humans with many of their essential needs, including life-saving pharmaceutical agents. In the last 6 years, five new

plant-derived drugs have been launched onto the market, and many more are currently undergoing clinical trials. As a vast proportion of the available higher plant species have not yet been screened for biologically active compounds, drug discovery from plants should remain an essential component in the search for new medicines, particularly with the development of highly sensitive and versatile analytical methods.

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