Plant-Derived Natural Products as Leads for Drug Discovery

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Li Pan, Esperanza J. Carcache de Blanco, and A. Douglas Kinghorn

Introduction

Natural product molecules having their origin from plants, microorganisms, and animals have had an irreplaceable role throughout the last 200 years in treating and preventing diseases, and continue to serve as important leads in modern drug discovery. Several volumes and reviews published recently have focused on this topic [1–6]. A considerable proportion of the natural products used as drugs is derived from terrestrial plants, which offer an invaluable and still incompletely exhausted resource for this purpose. In addition, profound ethnomedical knowledge based on the use of medicinal plants by humans has been accumulated for thousands of years. In the last few decades, pharmaceutical research on plants has been facilitated by the development of relevant technologies including new isolation methods, more sensitive spectroscopic techniques for structural determination, as well as specific high-throughput bioassay systems. Plant-derived bioactive compounds, in addition of being developed directly as drugs, also serve as prototype drug molecules, known as "lead compounds", and as pharmacological probes, to help better understand biochemical and physiological mechanisms.

In this chapter, some successful drugs derived from plant secondary metabolites in their original or modified forms, and other substances, currently under clinical trial as drug candidates, as well as several additional compounds used as biochemical probes afforded from plants, will be described. A general description of the importance and perspective of plants in drug discovery will also be given.

Role of Plants in Drug Development

Medicinal plants have been used as a major source of drugs for thousands of years in human history, and even today they are basis of the systematic traditional medicine practices in many countries all over the world. The first recorded literature on medicinal plants can be traced back to an earlier age of human history, such as the Atharvaveda (2000 BC) in India, the Divine Farmer's Herb-Root Classic (3000 BC) in China, and the Eber Papyrus (1550 BC) in Egypt [7, 8]. It is evident that the modern drug industry has been developed to a considerable degree as a result of plant-based traditional medicines. A review published in 2001 indicated that 88 active compounds isolated from 72 medicinal plants have been introduced into

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modern drug therapy, with many of them being considered as the active principle responsible for their ethnopharmacological use [9]. Some of these plant-derived therapeutic agents, such as atropine (anticholinergic), codeine (cough suppressant), colchicine (antigout), ephedrine (bronchodilator), morphine (analgesic), pilocarpine (parasympathomimetic), and physostigmine (cholinesterase inhibitor) are still being widely used today [10].

A whole new era for drug discovery opened up in the early nineteenth century, triggered by the isolation of morphine, a pharmacologically active compound from the plant medicine opium. Later, compounds from plants such as atropine (*Atropa belladonna*), cocaine (*Erythroxylum coca*), ephedrine (*Ephedra* spp.), digitoxin (*Digitalis purpurea*), and quinine (*Cinchona* spp.) were purified and then served as drugs [2, 4, 5]. These discoveries are considered important not only for introducing new single chemical entities as potent medicinal treatments, but also for helping understand human diseases by disclosing the key role that these molecules play, and by promoting the development of pharmacology, medicinal chemistry as well as organic chemistry [8]. As a result of the further purification of drugs such as artemisinin, digoxin, paclitaxel, vinblastine, and vincristine from plants in the twentieth century, the use of plant extractives in prescriptions such as tincture of belladonna was gradually replaced by pure single chemical entities like atropine.

Plants and other organisms may be regarded as libraries of small-molecule secondary metabolite organic compounds with considerable structural diversity, which would otherwise probably be unavailable in a synthetic chemical laboratory [11–14]. As secondary metabolites, these compounds have been elaborated in living organisms by complex enzyme systems developed during a long evolutionary process. It is apparent that these natural products present more "drug-like" or "biologically friendly" molecular qualities than many purely synthetic compounds. These intrinsic properties provide plant-derived small organic molecules and other natural products with an important potential role in modern drug discovery [15, 16].

Although combinatorial chemistry has been employed in the pharmaceutical industry over the last 2 decades to satisfy the need for very large numbers of compounds demanded in high-throughput screens (HTS), the results have not been as promising as expected, and the number of new chemical entities introduced annually as produced by this method has actually declined [3, 17]. A combination of natural products and combinatorial chemistry has been initiated in recent years. In this case the latter serves as a technique to optimize the structure of existing active natural compounds to new agents [17–20].

Active compounds isolated from plants can serve directly as therapies in clinical use, like morphine, atropine, quinine and paclitaxel, or as prototype biologically active "lead" compounds, affording numerous structural analogs as new pharmaceutical agents, such as artemisinin and the opiate derivatives. In addition to their medicinal use, some secondary metabolites from plants have also served as powerful "pharmacological tools" to help explain the mechanisms underlying human diseases [21–24]. Today, drug discovery from plants is based mainly on bioactivity-guided isolation, and groups of scientists with different research backgrounds including botany, biochemistry, pharmacology, pharmaceutics, pharmacognosy, medicinal chemistry, organic chemistry and toxicology are required in this enterprise [12–14, 25, 26].

Plant Natural Products as Drugs

Despite the "synthetic revolution" in the pharmaceutical industry, medicinal plants are still involved in the primary health care of a large proportion of the population in the world, especially in developing countries [9]. Bioactivity-guided

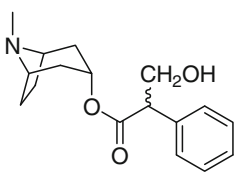
fractionation can often lead to the isolation of active principles of these medicinal plants, and some of those chemical entities with acceptable pharmaceutical qualities can be developed as drugs in their original forms directly. These include compounds on the therapeutic market that have been used for many years as important clinical agents mainly in the treatment of cancer, central nervous system disorders, cardiovascular diseases, and infectious diseases [11–14, 20]. In this section, some important plant-derived drugs in their unmodified forms will be mentioned, with a brief description about the plant origin and pharmaceutical use in each case.

Atropine (**1**) [a racemic mixture of (+)- and (-)-hyoscyamine (**2**)] and scopolamine [(-)-hyoscine] (**3**) are tropane-type alkaloids found in certain plants in the Solanaceae (nightshade) family used medicinally for centuries in Europe, such as *Atropa belladonna*, *Hyoscyamus niger*, and *Datura stramonium* [7]. The antispasmodic activities of atropine are due to competitive antagonism of acetylcholine at the muscarinic receptor site. Scopolamine is also an anticholinergic agent, and most commonly used for the prevention of nausea and motion sickness in the form of a transdermal patch. Both of these tropane alkaloids have psychoactive effects as a result of their ability to penetrate the blood-brain barrier [7, 27, 28].

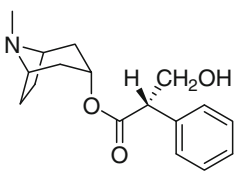
Nicotine (**4**), an agonist on the nicotinic acetylcholine receptor (nAChR) found in *Nicotiana tabacum* (tobacco), is used pharmaceutically for smoking cessation [29]. Analogs of nicotine are considered promising for the treatment of neurodegenerative conditions like Alzheimer's disease [30, 31].

Morphine (**5**) and codeine (methylmorphine) (**6**), two major morphinan-type alkaloids with an isoquinoline skeleton, are extracted from opium, the dried milky sap released from the immature fruits of poppies (*Papaver somniferum*). Morphine and codeine can interact with opioid receptors distributed in brain tissues and the periphery, and are most widely used as narcotic analgesics, with codeine also having an antitussive effect [4, 25, 32].

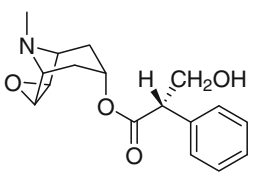
Galantamine (**7**, Razadyne®, Reminyl®, Nivalin®) is a recently approved drug for the treatment of early-onset Alzheimer's disease [12]. Galantamine (or galanthamine) is an Amaryllidaceae-type alkaloid first purified from the snowdrop (*Galanthus woronowii*) in



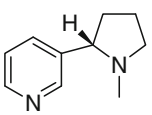
1. atropine



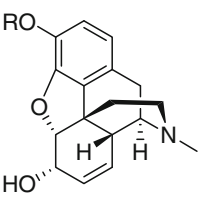
2. (-)-hyoscyamine



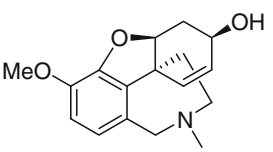
3. scopolamine
[(-)-hyoscine]



4. nicotine



5. R = Me codeine
6. R = H morphine



7. galantamine

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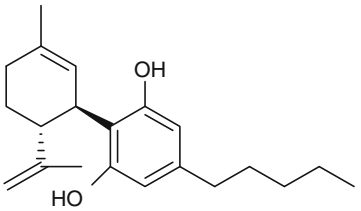
the early 1950s, and later found in other plants of the family Amaryllidaceae [34]. Galantamine can improve cerebral function by acting as a cholinergic agent, and inhibits acetylcholinesterase and modulates nicotinic acetylcholine receptors (nAChRs) [33–36]. The market need for galantamine is now met by the total synthesis of this compound [36].

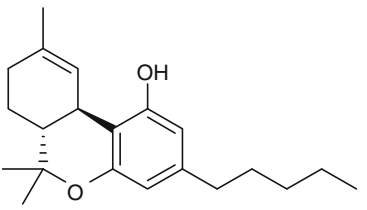
The demand for the legalization of *Cannabis sativa* (marijuana) for medicinal use has represented an interesting controversy in recent years because of the possibility of abuse [37]. Cannabidiol (CBD, **8**) and Δ^9-*trans*-tetrahydrocannabinol (THC, **9**), two active cannabinoids of marijuana, have been approved recently in Canada as ingredients of an oromucosal spray marketed as Sativex® to alleviate the pain caused by multiple sclerosis (MS). Efforts are being made to introduce Sativex® to other countries in the near future [38, 39].

Quinine (**10**) and quinidine (**11**), two diastereomeric alkaloids containing quinoline rings in their molecules, are obtained from *Cinchona* spp. and other species. Quinine (**10**) was the first effective treatment for falciparum malaria, an often fatal parasitic disease caused by several species of plasmodium. The discovery of quinine relieved European settlers from the harmful effects of this fatal illness, and greatly facilitated colonization in many tropical and subtropical areas of the world [40]. Quinine exerts its activity by inhibiting the heme polymerase of the parasitic host, and still shows some efficacy today as an antimalarial agent in cases where synthetic drugs fail due to parasite resistance [2, 6, 41]. Quinidine (**11**), has some use as a cardiac antiarrhythmic by affecting ion channels [2, 41].

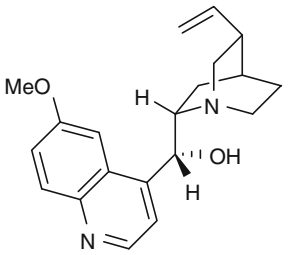
Artemisinin ("qinghaosu") (**12**), a sesquiterpene lactone possessing an unusual endoperoxide bridge, is a compound discovered in the People's Republic of China from *Artemisia annua*, which has long been used as a traditional medicinal plant for the treatment of fever. As a naturally occurring antimalarial, artemisinin may be employed as an option for the treatment of chloroquine-resistant malaria in China and some



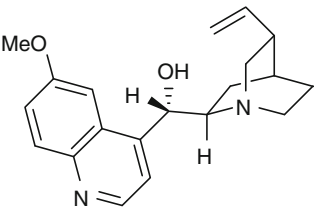
8. cannabidiol (CBD)



9. Δ^9-*trans*-tetrahydrocannabinol (THC)



10. quinine



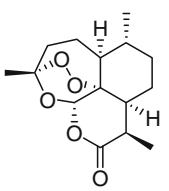
11. quinidine

other countries in Asia [42]. Artemisinin exerts its activity through a unique mechanism by acting on the heme complex [43]. However, the use of artemisinin as a monotherapy antimalarial agent is no longer recommended, since this might lead to parasite resistance to this entire compound class [44].

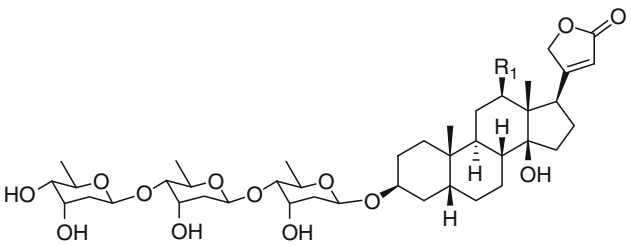
In the nineteenth century, digitoxin (**13**), a major cardioactive steroid glycoside, was isolated from *Digitalis purpurea*, commonly known as purple foxglove. This plant proved to be an effective treatment for dropsy caused by congestive heart failure in the late eighteenth century in England [45]. Digoxin (**14**) is another active cardiotonic glycoside that was purified from *Digitalis lanata* subsequent to the discovery of digitoxin. These two drugs exhibit a positive inotropic effect by inhibiting the activity of ATPase and cation transport, thus resulting in the increase of Ca^{2+} levels in the myocytes [46].

Paclitaxel (**15**, Taxol®) is a diterpenoid based on the taxane nucleus, possessing an essential oxetane ring and with one of the substituent groups containing a nitrogen atom. This compound was first isolated from the bark of *Taxus brevifolia* (Pacific yew) [47]. As a chemotherapeutic anticancer agent, paclitaxel inhibits mitosis by acting as a microtubule stabilizer and has been used in the clinic primarily for the treatment ovarian cancer, breast cancer, and non-small cell lung cancer [48, 49]. The limited availability of the plant source of paclitaxel was once a considerable obstacle in the development of this drug, until new semisynthetic and biological methods were developed to solve this problem, as will be discussed later in this chapter.

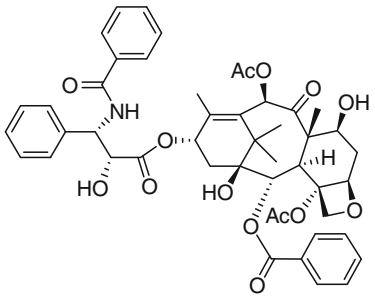
Vinblastine (**16**, Velban®, Alkaban-AQ®) and vincristine (**17**, Oncovin®) are structurally closely related indole-dihydroindole dimers (bisindole alkaloids), isolated from *Catharanthus*



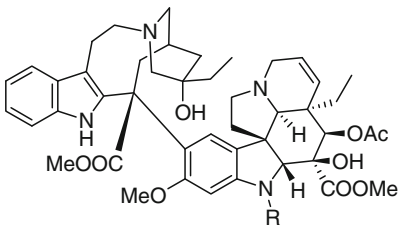
12. artemisinin



13. R_1 = H digitoxin
14. R_1 = OH digoxin



15. paclitaxel (taxol)



16. R = CH_3 vinblastine
17. R = CHO vincristine

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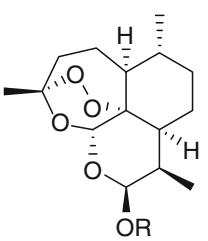
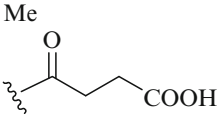
roseus, also known as *Vinca rosea* (Madagascar periwinkle). Vinblastine and vincristine are used mainly to treat different forms of leukemia, although they have efficiency against several other forms of cancer. Those two anticancer agents act as mitotic inhibitors by binding to dimeric tubulin, and then leading to the failure of microtubule assembly in the metaphase stage [50–52].

Semi-synthetic Drugs Based on Plant Secondary Metabolites

The use of semi-synthetic derivatives as drugs developed from plant natural product lead compounds began at the end of the nineteenth century and may be exemplified by drugs like aspirin (acetyl salicylic acid). From then on, the potential of plant-derived compounds as leads in drug discovery has been realized on numerous occasions [8]. A survey released recently revealed that over 40% of the new small-molecular single chemical entity drugs introduced to the market from 1981 to 2006 are natural product derivatives, with 28% of them being chemical semi-synthetic modifications based on natural products, 12% "natural product mimics", and 12% synthetic compounds in which the pharmacophore modeled was that of a natural product [20]. Synthetic optimization of the naturally occurring compounds is often required to enhance therapeutic potency, to increase bioavailability, to remove unpleasant side effects, and to help compensate for a shortage in a natural product drug supply, or to derive different bioactivities. This section will focus on examples of semi-synthetic drugs of plant origin.

A series of artemisinin-based semisynthetic antimalarial derivatives, with all of them maintaining the key endoperoxide bridge, such as arteether (**18**), artemether (**19**), artesunate (**20**), and dihydroartemisinin (**21**), have been designed to improve the water solubility and the metabolic stability of artemisinin [53, 54]. Among them, dihydroartemisinin (artenimol), is considered as a common active metabolite of artemisinin derivatives [53, 54]. Currently, artemisinin-based therapies combined with standard antimalarials such as amodiaquine, sulfadoxine-pyrimethamine, mefloquine, and lumefantrine are recommended by the World Health Organization (WHO) as first-line therapies for malaria [55, 56].



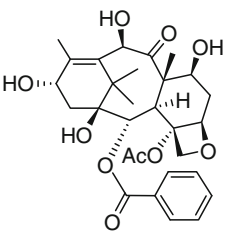
18. R = Et arteether
19. R = Me artemether
20. R =  artesunate

21. R = H dihydroartemisinin

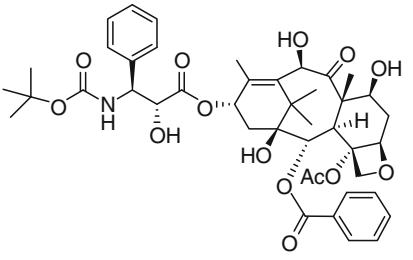
As mentioned before, the market demand of the anticancer drug, paclitaxel (**15**, Taxol®) was threatened initially by an unsustainable natural supply, which relied mainly on extracting this compound from the bark of the slow-growing Pacific yew tree, from which it is produced only in a very low yield [57]. Although the total synthesis of paclitaxel has been described, this is still inefficient when considered the extensive market need [58–60]. 10-Deacetylbaccatin III (**22**), which is available with a relatively high yield from the leaves of other renewable yew species, such as *Taxus baccata* L., was introduced as a semi-synthetic precursor compound of paclitaxel by the major pharmaceutical manufacturer, Bristol-Myers Squibb (B-MS) [61]. Docetaxel (**23**), a related taxane anticancer drug can also prepared from 10-deacetylbaccatin III (**22**) [62].

Oseltamivir phosphate (**24**, Tamiflu®), a neuraminidase inhibitor, is administered orally for the treatment and prevention of influenza A and B virus infections, and stockpiling of this drug has been proposed to counter the threat of a pandemic such as avian flu [63, 64]. One synthesis of this compound begins from (−)-shikimic acid (**25**), which serves as the key intermediate in the biosynthesis of a variety of aromatic compounds in plants and microorganisms [2]. Shikimic acid was first isolated from the plant *Illicium anisatum* (Japanese "shikimi") in 1885, and is abundant in the star aniseed (*Illicium verum*) and the plant genus *Liquidambar* ("sweetgum") [65, 66]. In order to overcome the shortage of the natural source of shikimic acid, fermentation of this substance by metabolically engineered *Escherichia coli* strains has been developed successfully as an efficient alternative method of production [67]. Recently, a total synthetic method for oseltamivir phosphate independent of shikimic acid has been developed [68].

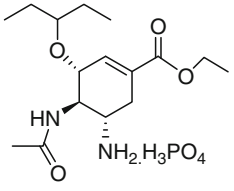
Nitisinone (**26**, Orfadin®) is the first drug approved in Europe for the treatment of hereditary tyrosinemia type 1 (HT-1), a rare genetic metabolic disorder caused by a deficiency of fumarylacetoacetate hydrolase (FAH), an enzyme involved in the metabolism of tyrosine [69]. Nitisinone is a derivative of leptospermone (**27**), an effective herbicide present in the bottlebrush



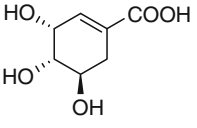
22. 10-deacetylbaccatin III



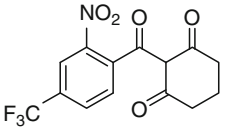
23. docetaxel



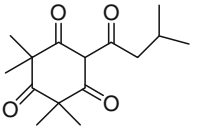
24. oseltamivir phosphate



25. (-)-shikimic acid



26. nitisinone

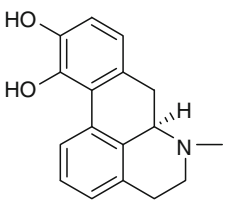


27. leptospermone

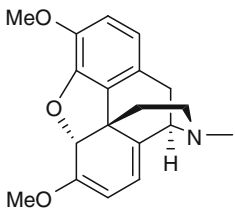
plant *Callistemon citrinus* [70]. Nitisinone can interfere with tyrosine catabolism as a competitive inhibitor of 4-hydroxyphenyl pyruvate dioxygenase (HPPD), an upstream enzyme of FAH, to prevent accumulation of toxic tyrosine metabolites that lead to liver and kidney failure [69–71].

Opiates, narcotic compounds extracted or derived from opium, are a remarkable source of lead compounds for their potent pharmaceutical effects such as analgesics, antitussives and ataractics, and of which many synthetic derivatives have been prepared [8, 72]. Apomorphine (**28**), a dopamine agonist derivative from morphine (**5**) but without analgesic properties like morphine, was recently approved as a therapy for Parkinson's disease [73]. Hydrocodone (**30**) is a narcotic agent derived from thebaine (**29**) and is commonly combined with other analgesics such as acetaminophen and ibuprofen as drugs to relieve pain. [74]. Naloxone (**31**) and naltrexone (**32**) are both opioid receptor antagonists. Naloxone is used as a treatment for opioid overdose [75], while naltrexone can be used to treat alcoholism as well as opiate addiction [76]. Dextromethorphan (**33**) is a non-narcotic opiate derivative that is widely used as an over-the-counter cough-suppressant [77].

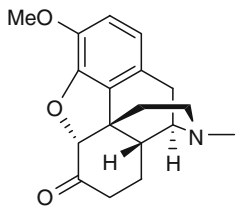
Podophyllotoxin (**34**), is a lignan constituent of *Podophyllum* resin, produced from the North American mayapple (*Podophyllum peltatum*) and the Himalayan species *Podophyllum hexandrum* [78, 79]. As a result of its considerable toxicity, extensive chemical modification work has been done to improve the pharmaceutical profile of podophyllotoxin. Etoposide (**35**), teniposide (**36**), and etopophos (etoposide phosphate, the prodrug of etoposide) are successful anticancer drugs derived from podophyllotoxin [79]. Instead of acting as microtubule inhibitors in the same manner as the lead compound **34**, derivatives **35** and **36** exert their anticancer activities by acting as inhibitors of the enzyme topoisomerase II, and together are widely used to treat lymphomas, acute leukemia, small cell lung cancer, and testicular cancers [78–80].



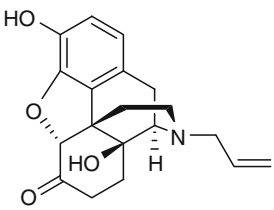
28. apomorphine



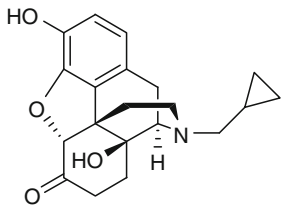
29. thebaine



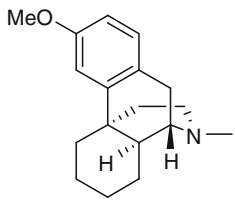
30. hydrocodone



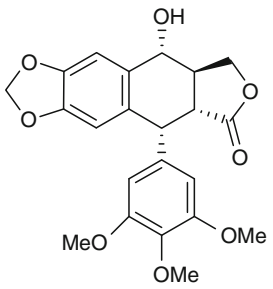
31. naloxone



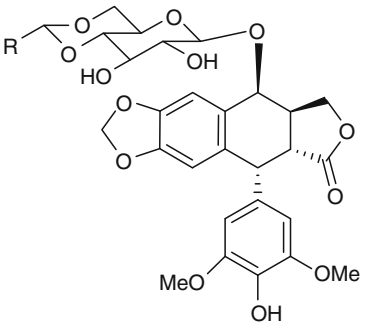
32. naltrexone



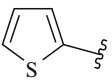
33. dextromethorphan



34. podophyllotoxin



35. R = Me etoposide

36. R =  teniposide

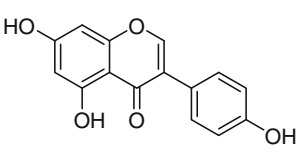
Use of Plant-Derived Natural Products as "Pharmacological Probes"

A number of plant secondary metabolites have been found to be valuable "pharmacological probes" or "biochemical tools" to help target various receptors as well as to explain cellular processes and assist with the elucidation of different kinds of molecular targets.

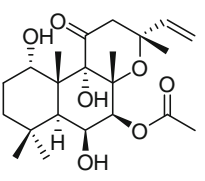
Genistein (**37**), an estrogenic isoflavone present in soybean (*Glycine max*), is a protein tyrosine kinase (PTK) inhibitor, and has been used as a probe to study the interaction between PTK and cyclic nucleotide-gated (CNG) channels [81, 82]. Genistein is currently under clinical trial for its angiogenesis-inhibiting activity [83].

Forskolin (**38**), also known as coleonol, is a labdane diterpene isolated from the roots of *Coleus forskohlii* (Lamiaceae), a traditional medicinal plant used in India for the treatment of heart disease and asthma [84]. As a hypertensive agent with spasmolytic, cardiotonic and anti-platelet-aggregation activity, forskolin can directly activate adenylate cyclase (AC), a target enzyme of multiple G-protein-coupled receptors (GPCRs), leading to the intracellular accumulation of cAMP [85, 86]. This property makes it a potent pharmacological tool widely used in investigating the catalytic mechanism of AC and the regulation of cAMP [86–88].

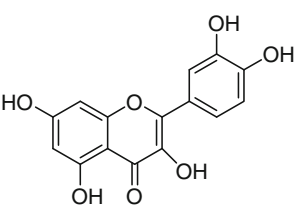
Quercetin (**39**) is one of the most common natural occurring flavonoids and is found in many fruits and vegetables, such as apples and onions. It is well known for its antiinflammatory, antioxidant, and antiplatelet aggregation activities [89, 91]. Quercetin can induce a wide spectrum of cellular events by interacting with a



37. genistein



38. forskolin



39. quercetin

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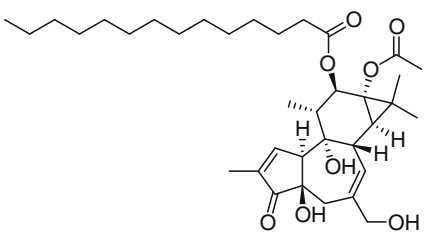
number of signaling molecules such as protein kinases, NF-κB, and calmodulin (CaM) [89–91]. This flavone can bind to intracellular targets, such as nucleic acids and proteins and exhibits specific fluorescence emission [92, 93]. This property allows quercetin to be a valuable, sensitive, and selective spectroscopic probe to trace phenol-binding complexes in cellular systems and help clarify the relevant molecular functions [92–94].

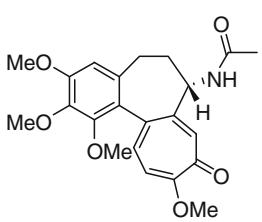
Phorbol esters, tetracyclic diterpenoids naturally occurring in some plants of the family Euphorbiacaeae, such as *Croton tiglium*, show protein kinase C (PKC) inhibition activity, and are also known for their skin-irritant and tumor-promotion properties [95, 96]. The most abundant *C. tiglium* phorbol ester derivative, 12-*O*-tetradecanoylphorbol-13-acetate (TPA) (**40**), is used as a standard tumor promoter in animal models of full-term carcinogenesis [97].

Some microtubule targeting agents like colchicine (**41**), as well as certain anticancer agents mentioned before, such as podophyllotoxin (**34**), paclitaxel (**15**), vinblastine (**16**) and vincristine (**17**), may be used as biological probes in cancer research. Thus, paclitaxel acts as a promoter of stabilization of microtubules [98–102].

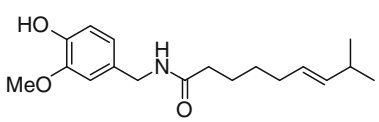
Capsaicin (**42**), a pungent principle in chili peppers (*Capsicum* spp.), as well as the skin irritant, resiniferatoxin (**43**), from the latex of *Euphorbia resinifera*, have been used to probe the transient receptor potential (TRP) channels and the vanilloid receptors responsible for the pain sensation caused by heat. This has led to the successful isolation of the first nociceptive receptor, TRPV1 (transient receptor potential channel, vanilloid subfamily member 1) [103, 104]. Capsaicin-containing creams are now available as nonprescription pain-relievers for the treatment of post-therapeutic neuralgia [105]. The internal use of capsaicin for the treatment of severe post-operative pain, post-traumatic neuropathic pain, and musculoskeletal diseases is currently under clinical trial [106].



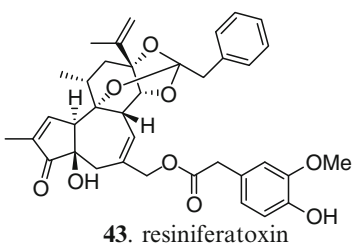
40. 12-*O*-tetradecanoylphorbol-13-acetate



41. colchicine



42. capsaicin



43. resiniferatoxin

Examples of Candidate Drug Molecules from Plants

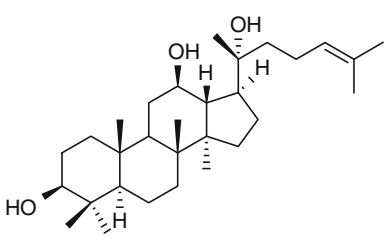
Although the development of new drugs from plants and other organisms has received less emphasis in the last few decades than previously, plants are still an invaluable resource of therapeutic leads, as judged by the number of chemical entities currently under preclinical or clinical trials [107–109]. A portion of these compounds are analogs developed on the basis of known drugs such as paclitaxel and vinblastine [107] and these will not be covered in the present section. Herein, several other examples of promising candidate drug molecules of plant origin will be described briefly.

Protopanaxadiol (**44**), a triterpene aglycone derived from several saponins of ginseng (*Panax ginseng*) [110], has been demonstrated as a potential anticancer agent with effects on apoptosis induction and cell cycle arrest on cancer cells both *in vitro* and *in vivo*, and has also been reported to show cytotoxicity against multidrug-resistant tumors by blocking P-glycoprotein (P-gp, MDR1) [111–114]. Protopanaxadiol, under the trade name Pandimex®, is now in a Phase I clinical study in the United States for the treatment of lung cancer and other solid tumors [111]. Also, it has been approved conditionally in mainland China for the treatment of advanced cancers of the lung, breast, pancreas, and colon-rectum [114].

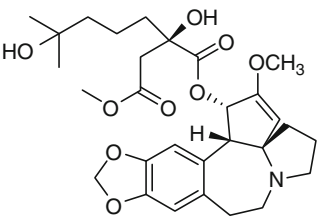
Homoharringtonine (Ceflatonin®, HHT) (**45**) is a cytotoxic alkaloid isolated from several plants in the genus *Cephalotaxus*, such as *C. harringtonia*. This compound exerts its potential antineoplastic activity by inhibiting protein synthesis at the ribosome level and inducing the differentiation and apoptosis of cancer cells [115–117]. Homoharringtonine is currently under II/III phase clinical trials for the treatment of patients with chronic myeloid leukemia (CML) in the United States and Europe [117, 118].

Oxymatrine (**46**) is one of the major alkaloids isolated from the aqueous extract of the roots of *Sophora flavescens*, a traditional Chinese medicinal plant mainly used in the treatment of jaundice and other liver disorders [119]. Oxymatrine has been developed in mainland China as an injection for the treatment of chronic hepatitis B virus (HBV) infection [120]. Clinical trials have shown the effect of oxymatrine against chronic hepatitis C (HCV) with the function of inhibiting proliferation, lessening liver fibrosis, regulating the immune reaction though reducing the level of TNFα (tumor necrosis factor alpha) in the serum, and down-regulating the expression of Fas receptor/Fas ligand in the liver tissue [121–123].

Phenoxodiol (**47**), derived from genistein (**48**), a natural occurring soy isoflavone, has been promoted as a new promising drug candidate in oncology for its broad anticancer spectrum and minimal toxicity [124]. Phenoxodiol exhibits its anticancer function by inducing the mitotic arrest and apoptosis of tumor cells through multiple mechanisms such as by decreasing the level of antiapoptotic proteins and acting as a DNA topoisomerase (topo) II



44. protopanaxadiol



45. homoharringtonine

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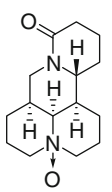
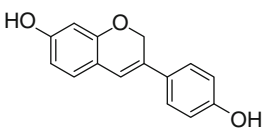
inhibitor [124, 125]. Phenoxodiol is now undergoing clinical trials as a therapy for ovarian and prostate cancer in the United States, Europe, and Australia [126, 127].

Huperzine A (**49**) is a sesquiterpene alkaloid isolated from a traditional Chinese herbal remedy, *Huperzia serrata* ("Qian Ceng Ta") in 1986 [128]. This compound has been proved to be a potent, selective and reversible acetylcholinesterase (AChE) inhibitor, and demonstrated memory enhancement and neuroprotective functions in clinical trials as a therapeutic against Alzheimer's disease (AD) in the People's Republic of China [129, 130]. In 2004, a phase II clinical trial focused on its cognitive function was initiated by the National Institute on Aging (NIA) in the United States [131]. ZT-1 (**50**), considered more selective than huperzine A, was developed as a semi-synthetic derivative of **49** by cooperation of the Shanghai Institute of Materia Medica and Debiopharm of Switzerland and is currently under phase I/II clinical trials in mainland China and in Europe [132].

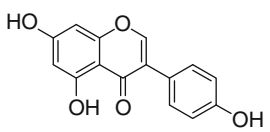
Betulinic acid (**51**) and its close structural anolog, betulin (**52**), are lupane triterpenoids widely distributed in the plant kingdom, with the latter especially abundant in the bark of the white birch tree [133]. Betulin can serve as the semi-synthetic precursor of betulinic acid, which is considered biologically more active than betulin. Important biological activities attributed to betulinic acid and its derivatives, are anti-HIV activity and selective cytotoxicity against melanoma cancer cells [133–135]. Betulinic acid can induce apoptosis by acting on the mitochondria of various tumor cells. This compound is currently under phase I/II clinical trials launched by National Cancer Institute (NCI) as a chemotherapeutic agent for the treatment of dysplastic melanocytic nevi, which are considered associated with cutaneous melanomas [136].



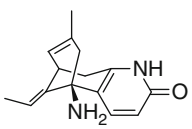
46. oxymatrine



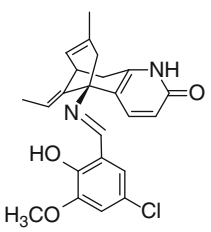
47. phenoxodiol



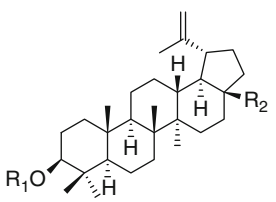
48. genistein



49. huperzine A



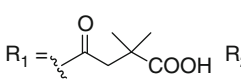
50. ZT-1



51. betulinic acid $R_1 = H, R_2 = COOH$

52. betulin $R_1 = H, R_2 = CH_2OH$

53. bevirimat



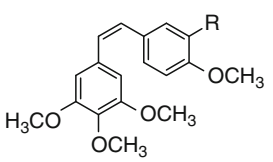
Bevirimat (PA-457, **53**) is a semi-synthetic analog of betulinic acid with potent and selective antiretroviral activity. As a first-in-class maturation inhibitor, bevirimat can inhibit the replication of HIV by interrupting the cleavage of the CA-SP1 (capsid-spacer peptide 1) in the Gag processing step, thus leading to defects in viral core condensation [137, 138]. Bevirimat is being developed as a new agent for the treatment of HIV infection, and phase II trials completed recently have suggested a favorable profile of safety and pharmacokinetics that warrant further clinical research [139, 140].

Combretastatin A4 phosphate (**54**, CA4P) is a water-soluble prodrug of the natural stilbenoid, combretastatin A4 (**55**), produced by the South African tree *Combretum caffrum* [141]. CA4P is the first tubulin-binding vascular targeting agent (VTAs) under clinical trial, and can induce morphological changes within endothelial cells and then led to vascular dysfunction in tumors [141, 142]. CA4P as a therapeutic agent for the treatment of anaplastic thyroid carcinoma and myopic macular degeneration is under separate phase II clinical trials [143].

Curcumin (**56**), a yellow polyphenol pigment isolated from turmeric (*Curcuma longa*), commonly used as a spice in cooking, has utility in India not only as a dietary supplement but also for healthcare [144]. It exhibits antioxidant, antiinflammatory, antimicrobial, and immunomodulatory activities as well as other effects germane to cancer. Curcumin regulates numerous intracellular signaling pathways by acting on a broad spectrum of targets including transcription factors, growth factors, inflammatory cytokines, and protein kinases [145]. The potential therapeutic effects of curcumin have raised interest worldwide and multiple clinical trials have been launched in different countries including India, Japan, the People's Republic of China, and the United States, for the treatment of cancer, Alzheimer's disease as well as chronic psoriasis [144].

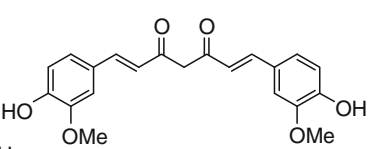
Future Prospects

The successful track record of natural compounds from plants, microorganisms, and other organisms, has demonstrated amply that these small organic molecules represent a highly useful source of molecular diversity in drug discovery [1, 5, 6, 9, 14]. Although the trend of developing small organic molecules as new drugs in the last 2 decades has been challenged by new synthetic methods such as combinatorial chemistry, natural products have once again come to prominence as sources of libraries of drug-like chemical entities [3, 18, 20]. Many small biotechnology companies as distinct from large pharmaceutical corporations have realized the important role that natural products play in modern drug discovery. Accordingly, new natural product-derived drugs are continually being introduced onto the market at a steady rate and there are numerous drug candidates from plants or other natural sources currently undergoing preclinical or clinical trials [107, 146]. Resourceful interdisciplinary efforts are required



54. combretastatin A4 phosphate R = OPO_3H_2

55. combretastatin A4 R = OH



56. curcumin

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to ensure that research on natural product drug discovery may keep pace with the many ongoing changes in the pharmaceutical industry.

The investigation of active compounds from plants and other organisms has benefited from many technological breakthroughs over the last 10 years. The utilization of bioassay-guided fractionation has been increased significantly by improving the technologies applied in the processes of compound analysis, purification, and structural identification as well as bioactivity screening. Thus, methods such as HPLC-coupled spectroscopy, higher magnetic field-strength NMR instruments, and robotics to automate high-throughput bioassays have all served to make the lead selection phase of plant-derived drug discovery faster and more reliable.

The combination of HPLC or LC with other techniques such as the diode-array detector (DAD), circular dichroism (CD), mass spectrometry (MS), and nuclear magnetic resonance (NMR) has tremendously increased the ability of analysis and purification of HPLC by providing structural information of compounds on-line with minimum quantities of samples. These upgrades in the use of HPLC have made the structural characterization of compounds in crude natural product mixtures more accurate and their isolation more straightforward [147–149].

Contemporary natural product structure elucidation depends largely on the sensitivity of NMR spectroscopy, which has been increased greatly by recent developments in NMR probe technology. The introduction of microprobes and cryogenically cooled probes in NMR spectroscopy has afforded a considerable increase in sensitivity compared to conventional NMR probes and now enables the structure elucidation of compounds at the microgram level. The development of flow-through probes has provided a seamless link of NMR spectroscopy with liquid chromatography systems, and this promising technique has been adopted in natural products research [150, 151]. Accordingly, improvements to solvent suppression techniques have made it feasible to use non-deuterated solvents instead of expensive deuterated solvents during chromatographic separation and the LC-SPE-NMR technique can also make deuterated solvents unnecessary during the chromatographic separation, using the solid-phase extraction (SPE) technique before NMR analysis [152, 153].

Bioactivity screening is a key step in natural product-derived drug discovery. Over the last decade, numerous efforts have been carried out to develop more efficient screening methods. Thus, improved automated high-throughput techniques have allowed for rapid screening of plant extracts in the same manner as libraries of pure compounds, so the biological assays are no longer a rate-limiting step in the drug discovery process. With advanced data handling systems and robotics, a hundred thousand samples can be tested in just over a week [154]. New technologies based on bioactivity combined with chemo-analytical processes have emerged and some of them have been practiced successfully in plant-based drug research. These new methods, such as bioautography, HPLC-based activity profiling, on-flow bioassays, assays based on capillary electrophoresis, molecular imprinted polymers, biosensors, biological chip-based technologies for affinity separation and expression profiling, and various MS and NMR-based methods, have led to the establishment of effective, flexible and selective approaches for successful secondary metabolite screening from organisms [155, 156].

Biotechnology may be employed to overcome the sourcing problems that are unavoidable obstacles in the process of plant-derived drug manufacturing. A plant natural product can be produced via cell culturing of the source organism or via genetic engineering in a heterologous host. The metabolic pathway in the organism can be modulated and transformed using chemical or biological methods to furnish certain plant secondary metabolites of interest [157–160]. For example, since 2002, Bristol-Myers Squibb has produced

paclitaxel using callus cell cultures of the Chinese yew, *Taxus chinensis* [161]. Other plant-derived compounds such as some *Catharanthus* alkaloids, diosgenin from *Dioscorea* species, and the *Panax ginseng* ginsenosides also can be produced by cell culture [162–164]. These biotechnological methods have allowed selected plant natural products to be produced in a relatively controlled manner, and hence provide a supply of plant matrix not limited by sourcing problems, such as environmental, seasonal, geographical, and political factors [160].

The past history of use plant and other natural product-derived drugs in the treatment of many major afflictions like cancer, cardiovascular diseases, and neurological conditions augurs well for their future utilization in this regard. When considering plant sources specifically, no more than 20% of the existing higher plants on earth have been investigated for pharmaceutical purposes [146]. Moreover, even a large proportion of the known compounds with plant origin have never been evaluated in a bioassay [146]. Cooperative efforts from all the technical disciplines related to drug discovery should be continued to make plant-derived natural products research an essential contributor in the future. Towards this end, a greater effort than previously should be made to examine the traditional practices of plants in developing countries, and plant-derived compounds should be examine for efficiency in test systems germane to a wide spectrum of human and animal diseases than previously.

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