Current Developments in the Discovery and Design of New Drug Candidates from Plant Natural Product Leads†,‡

Kuo-Hsiung Lee*

Natural Products Laboratory, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7360

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This review article will emphasize recent research in the Natural Products Laboratory, School of Pharmacy, University of North Carolina at Chapel Hill, on various classes of plant-derived compounds that possess potent antitumor or anti-HIV activity. These compounds were obtained by bioactivity- and mechanism of action-directed isolation and characterization coupled with rational drug design-based modification and analogue synthesis. Structural modification, SAR, and mechanism of action studies are discussed.

From ancient to modern times, herbs and other plants have been used as medicinal agents, first only on a folkloric basis and later developed on a scientific basis into single agent drugs, such as the antiasthmatic drug ephedrine from *Ephedra sinica*.[1] The drug discovery and development program in the author's Natural Products Laboratory (NPL) and those of other researchers worldwide use plants as an essential route to new pharmaceutical leads.[2]

A major objective of the NPL is the preclinical development of bioactive natural products and their analogues as chemotherapeutic agents, including plant-derived antitumor agents, particularly novel etoposide analogues, anti-HIV compounds and analogues, as well as antimalarial, antifungal, antiviral, and anti-inflammatory agents. Chinese herbal medicine is the primary, although not sole, source of new leads. The three main research approaches are (a) bioactivity- or mechanism of action-directed isolation and characterization of active compounds, (b) rational drug design-based modification and analogue synthesis, and (c) mechanism of action studies. Drug discovery is an iterative process of lead discovery (i.e., isolation of bioactive lead compound(s) from these natural sources) coupled with lead improvement (rational design and synthesis of new analogues to improve pharmacological profiles). After selection of a new lead, drug development continues outside of the academic laboratories through preclinical studies (toxicology, formulation, and production) followed by clinical trials. In the academic laboratory, drug design/structure modification employs several tools to identify the optimum chemotherapeutic agent:

(a) structure−activity relationship (SAR) studies including both qualitative and quantitative SAR,

(b) mechanism of action studies including drug receptor interactions and specific enzyme inhibitions,

(c) drug metabolism studies including identification of bioactive metabolites and blocking of metabolic inactivation,

(d) molecular modeling studies including determination of 3D pharmacophores,

(e) combinatorial chemistry, including creation of peptide and nonpeptide libraries to generate new leads.

The discussion below will present several of these agents as well as prior and new lead identification and research performed in the NPL.

Plant-Derived Antineoplastic Agents and Their Analogues as Clinical Anticancer Drugs (Figures 1−3)

Many plant antitumor agents are used as clinical anticancer drugs: in the United States (in chronological order) vinblastine (Velban, **1**), vincristine (Oncovin, 2), vinorelbine (Navelbine, 3), etoposide (VP-16, **4**), teniposide (VM-26, 5), paclitaxel (Taxol, **6**), docetaxel (Taxotere, **7**), topotecan (Hycamtin, 8), and irinotecan (Camptosar, **9**) and many others in China, including 10-hydroxycamptothecin (**10**) and homoharringtonine (**11**) (Figure 1).

Vinblastine (**1**) and vincristine (2) are major drugs used to treat Hodgkin's lymphoma and acute childhood leukemia. They are natural alkaloids isolated from *Catharanthus roseus* (L.) G. Don (Apocynaceae) or *Vinca rosea* L. (Chinese medicine: "Chang Chung Hua").[3] Among numerous synthetic analogues designed to have activity against other tumor types or fewer side effects, Navelbine (**3**) (vinorelbine) was developed by Burroughs Wellcome and is used against non-small cell lung and advanced breast cancers.[4,5] This compound is a synthetic analogue of **1**, but has a nine-membered rather than an eight-membered C ring and a dehydrated D ring.[4,6,7]

The Chinese tree *Camptotheca acuminata* Decne. (Nyssaceae)[8] contains the natural anticancer alkaloids camptothecin (**12**) (Figure 2) and 10-hydroxycamptothecin (**10**),[8] which are used to treat gastric, rectal, colon, bladder, liver, and head and neck cancers in the People's Republic of China. However, synthetic derivatives, including topotecan (8)[9,10] and irinotecan (**9**, CPT-11),[11−13] were developed to answer water solubility problems of the natural alkaloids. They contain amino groups that can form more soluble hydrochloride salts; for example, compound 8 is 100-fold more water-soluble than **10**. All natural and synthetic camptothecins exert their anticancer activity by inhibiting the enzyme topoisomerase I (topo I). The NPL synthesized a series of water-soluble 7-(acylhydrazono)formyl camptothecins.[14] Compound **13** with a 7-(*l*-tyrosylhydrazono) group and four others were more potent than **12** in inhibiting DNA topo I and causing protein-linked DNA breaks, but were less toxic in several cancer cell lines.

Similarly, the NPL also chemically linked camptothecin (**12**) with paclitaxel (**6**), a natural antimitotic anticancer agent, through a variable length ω-aminocarboxylic acid

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* To whom correspondence should be addressed. Tel: 919-962-0066. Fax: 919-966-3893. E-mail: khlee@unc.edu.

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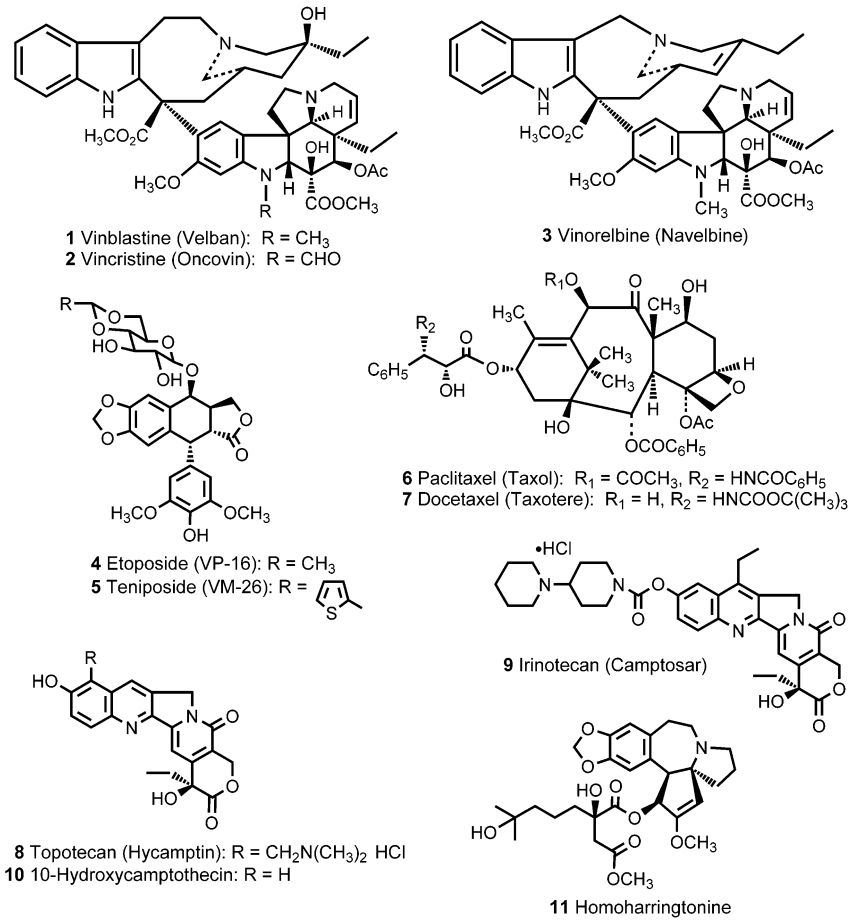

Figure 1. Clinical anticancer drugs (**1**−**11**).

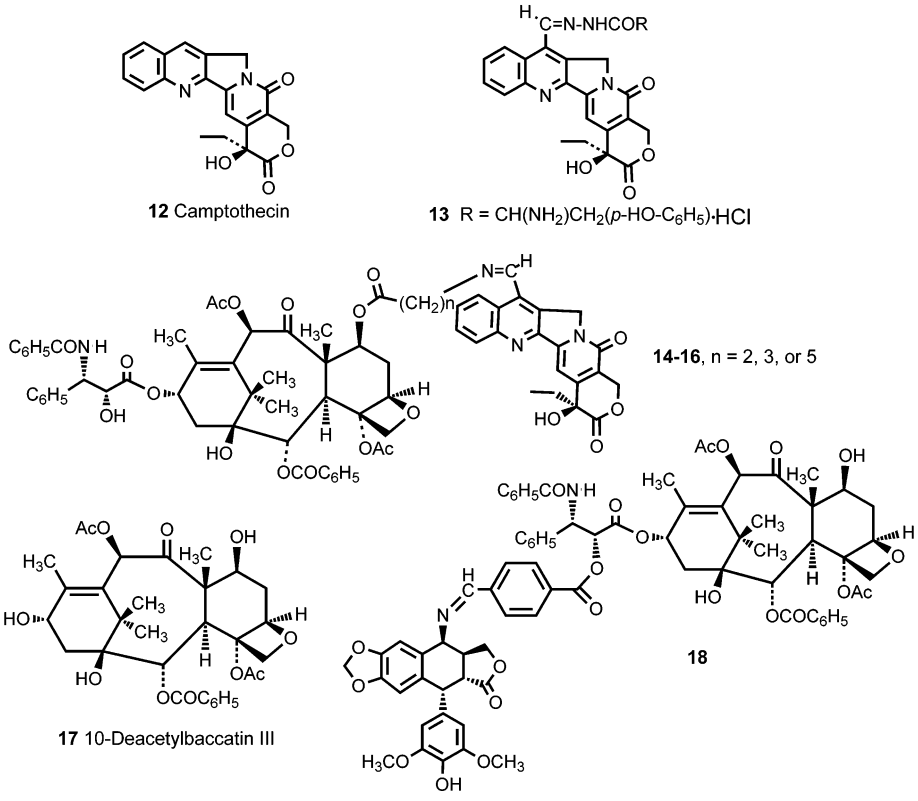

Figure 2. Camptothecin and paclitaxel analogues.

(imine bond to 7-formylcamptothecin and ester bond to paclitaxel's C-7 hydroxyl).[15] The resulting compounds (**14**−**16**) were less active than camptothecin against topo I, but still were potent inhibitors of tumor cell replication. In most cell lines, the conjugate compounds were more active than camptothecin, but less active than paclitaxel. However,

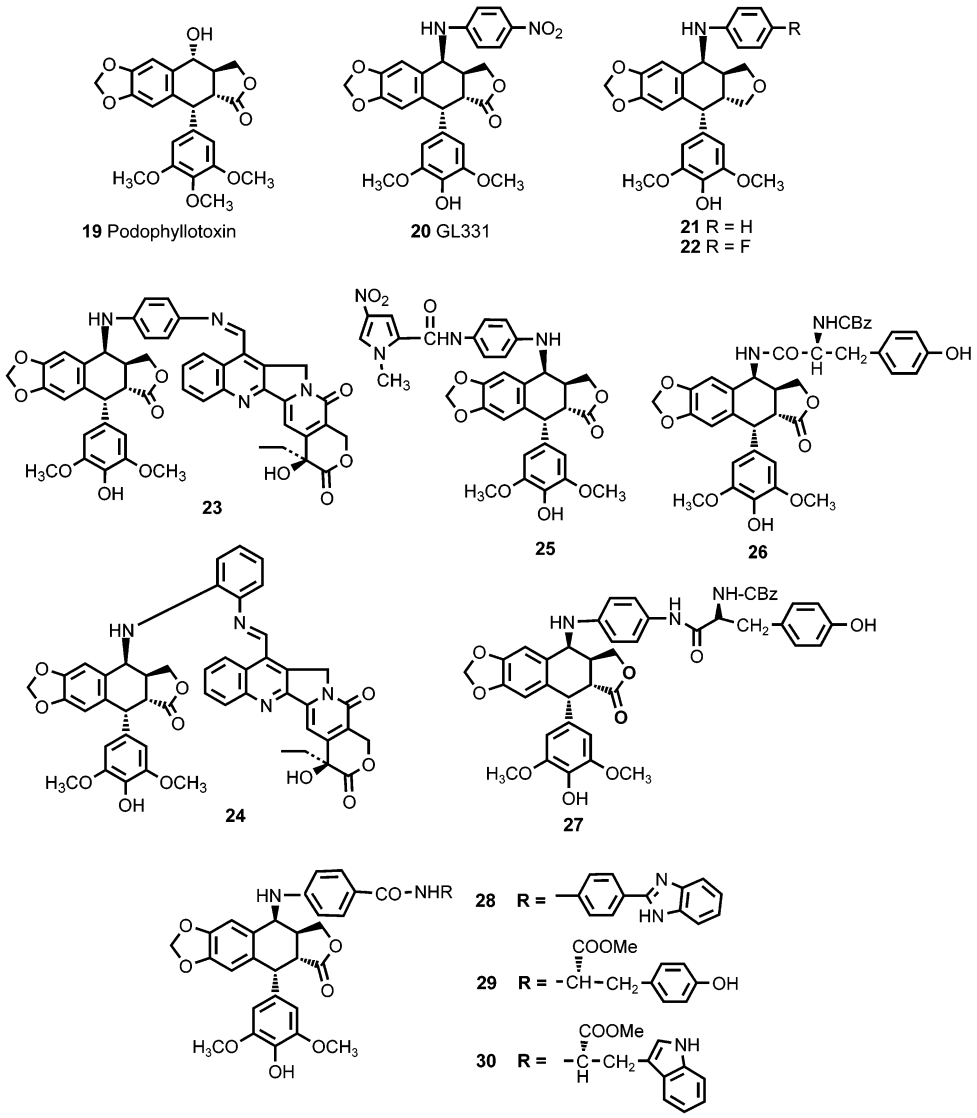

Figure 3. Etoposide analogues.

they were more active than either natural compound against HCT-8 cells, implying a possible novel mechanism of action.[15]

Paclitaxel (**6**) (Taxol), which is found naturally in the bark of *Taxus brevifolia* Nutt. (Taxaceae),[16] has a unique mode of anticancer action (it promotes the assembly of microtubules and consequently inhibits mitosis) and is active against breast, brain, tongue, endometrial, and ovarian cancers.[17−19] Because the natural source was nonrenewable and in scarce supply, analogue syntheses were widely pursued.[20−22] The clinically used docetaxel (**7**) is synthesized from the more readily available 10-deacetylbaccatin III (**17**), which is found in the needles of the European yew tree (*Taxus baccata* L.).[23] The NPL also conjugated paclitaxel with 4-aminoepipodophyllotoxin (see next section), using 4-carboxybenzaldehyde as the linker between the two compounds (imine bond to the 4-amino of epipodophyllotoxin and ester bond to the C-2′-hydroxyl of paclitaxel).[24] Compared with epipodophyllotoxin, the conjugate (**18**) showed comparable or better activity in several tumor cell lines and resulted in a lower-fold resistance in paclitaxel-resistant cell lines.

Etoposide Analogues. Podophyllotoxin (**19**) (Figure 3) is a natural lignan found in *Podophyllum pelatum* L. (Berberidaceae), *P. emodi* Wall, and *P. pleianthum* Hance. Like paclitaxel, podophyllotoxin is a mitotic inhibitor acting on tubulin; however, unlike paclitaxel, podophyllotoxin inhibits the assembly of microtubules. The clinical anticancer drugs etoposide (**4**) and its thiophene analogue teniposide (**5**) are semisynthetic analogues of podophyllotoxin. In these analogues, a β-C-4 glycosyl group has replaced the α-C-4 hydroxyl of podophyllotoxin and a hydroxyl has replaced the C-4′ methoxy group. Etoposide and teniposide do not affect mitosis, but instead inhibit the essential enzyme DNA topo II and, subsequently, increase DNA cleavage.[18] Although they are used clinically to treat small-cell lung cancer, testicular cancer, leukemias, lymphomas, and other cancers,[25−28] problems include poor bioavailability, myelosuppression, and drug resistance.[29] Consequently, the NPL, as well as other groups, has continued to explore structural modifications geared at improving pharmaceutical properties of the etoposide class, and the descriptions below illustrate many aspects of the drug development process, highlighted by the clinical trials (Phase II) with the semisynthetic analogue GL331 (**20**).

4-Amino-epipodophyllotoxin Derivatives Including GL331. On the basis of computer-modeling studies (see more details and studies below) and the possibility of forming water-soluble salts, the NPL synthesized numerous 4-alkylamino and 4-arylamino epipodophyllotoxin analogues from podophyllotoxin using a simple two-step reaction sequence.[30,31] Variously substituted arylamino analogues

showed comparable or greater % inhibition of DNA topo II activity and % cellular protein−DNA complex formation (DNA breakage)[30−32] and, more notably, greater cytotoxicity in etoposide-resistant cell lines. The optimal lead compound was GL331 (**20**),[33] which contains a *p*-nitroanilino moiety at the 4β position of **4**. Genelabs Technologies, Inc. has patented this technology and proceeded with clinical trials. The Phase I clinical trials as an anticancer drug were performed at the M.D. Anderson Cancer Center.[34] Initial results were quite promising: from marked antitumor efficacy in four tumor types (non-small and small cell lung, colon, and head/neck cancers), side effects were minimal with cytopenias being the major toxicity, and the maximum tolerated dose (MTD) was 300 mg/m^2. Phase II clinical trials against tumors other than gastric carcinoma, which is not sensitive to GL-331, are undergoing planning. GL331 and etoposide have the same major mechanism of action, inhibition of topo II; however, GL331 also has several advantages: (a) greater activity in vitro and in vivo, (b) more easily manufactured due to a shorter synthesis, and (c) activity against many multidrug-resistant cancer cell lines (KB/VP-16, KB/VCR, P388/ADR, MCF-7/ADR, L1210/ADR, HL60/ADR, and HL60/VCR).[34] Formulated GL331 shows desirable stability and biocompatability.[34] Overall, GL331 is an exciting chemotherapeutic candidate and a successful application of drug development from a natural products lead.

γ-Lactone Ring-Modified 4-Amino Etoposide Analogues. Metabolism of drug molecules can limit their efficacy by forming inactive compounds in vivo, for example, with etoposide the formation of inactive *cis*- and *trans*-hydroxy acids and epimerization to the *cis*-picrolactone. Thus, the NPL designed and synthesized γ-lactone ring-modified 4-amino epipodophyllotoxins. By replacing the lactone carbonyl with a methylene group, lactone hydrolysis and epimerization adjacent to the carbonyl should be eliminated. Two resulting compounds, unsubstituted- (**21**) and *p*-fluoro- (**22**) anilino analogues, did show comparable topo II inhibition (ID_{50} = 50 μM) and greater DNA breakage (125% and 139%, respectively, at 20 μM) than etoposide (100%, at 50 μM).[35]

Dual Topo I and Topo II Inhibitors. To circumvent the development of anticancer drug resistance, one plan is to simultaneously target two cancer processes, for instance, to target both topo I and topo II. This strategy has been implemented in compounds such as inotoplicine, a 7-*H*-benzopyrido[4,3-*b*]indole derivative. In the NPL, topo II-inhibitory analogues *p*-aminoanilino and an *o*-aminoanilino-substituted epipodophyllotoxins were chemically linked with a topo I-inhibitory compound, 4-formyl camptothecin, through an imine bond.[36] Compared with etoposide (**4**) [average log GI_{50} (M) = −5.01] and GL331 (**20**) [average log GI_{50} (M) = −5.9], the conjugate compounds **23** [average log GI_{50} (M) = −7.32] and **24** [average log GI_{50} (M) = −7.15] were significantly more cytotoxic in several cancer cell lines including HOP-62 leukemia, SW-620 colon cancer, MCF/ADR adriamycin-resistant breast cancer, and A-498 renal cancer. Also, **23** and **24** were more active than etoposide or camptothecin against corresponding drug-resistant KB cell lines and showed a lower-fold decrease in cytotoxicity (ca. 2- to 6-fold) than did etoposide (**4**) (80-fold) and camptothecin (**12**) (30-fold) in **4**-resistant (KB-7D) and **12**-resistant (KB-CPT) cell lines, respectively. In addition, compound **24** was less toxic in vivo (LD_{50} > 50 mg/kg/day) than either **4** or **12** when given i.p. to nude mice. Mechanistically, both compounds stimulated DNA cleavable complex formation with both topo I (although 2-fold less active than **12**) and topo II (compound **24**, but not **23**, was as active as **4**). Thus, conjugation of the two inhibitory components successfully produced dual topoisomerase inhibitors with cytotoxic activity against drug-resistant cells.[36,37]

CoMFA QSAR Study and Etoposide Analogues with Minor Groove Binding Enhancement. To construct an informative SAR model and improve analogue design, molecular modeling studies were conducted in the NPL[38,39] using comparative molecular field analysis (CoMFA) and CoMFA/q^2-GRS techniques to build three-dimensional quantitative structure−activity relationship (QSAR) models for 102 epipodophyllotoxins. Steric and electronic contour plots docked into DNA identified areas of positive and negative interactions and matched well with the composite pharmacophore model proposed in 1991 by MacDonald et al.[40] In addition, the CoMFA results suggested that if the minor groove binding ability of epipodophyllotoxin analogues is increased, the topo II inhibition should also increase. Several novel analogues were designed and synthesized, including a new compound (**25**) with a 1-methyl-4-nitro-2-pyrrolecarboxyl group, which is a structural component of the cytotoxic polypeptide netropsin.[41,42] This compound had lower log GI_{50} values in MOLT-4 leukemia and MCF-7 breast cancer cell lines (< −8 in both cell lines) than those of etoposide (**4**) (−5.99 and −5.36, respectively). In addition, compound **25** showed lower ID_{50}/LD_{50} values in KB cells [0.04/0.15 μM (**25**) vs 0.2/3.0 μM (**4**)] and was more active in topo II inhibitory activity [IC_{100} = 12.5 μM (**25**) vs 100 μM (**4**)] and protein-linked DNA breakage [225% (**25**) vs 100% (**4**) at 12.5 μM] assays.

***k*NN QSAR Study.** However, although CoMFA is a popular and relatively simple QSAR method, the calculated external predictive R^2 value for this study was below acceptable level and, thus, the CoMFA model was suggestive, but not predictive. In addition, as a 3D QSAR technique, CoMFA is sensitive to molecular alignment, which makes it nonreproducible. To overcome this problem, alternative computational techniques were explored and *k* Nearest Neighbor (*k*NN) QSAR, which uses 2D topological descriptors of chemical structures, was selected. *k*NN QSAR is a method based on the active analogues principle; that is, structural similarity leads to functional similarity and predicts the biological activity of a selected compound as the average of the activities of its *k* nearest neighbors. The NPL developed and applied *k*NN QSAR modeling to 157 epipodophyllotoxins.[43] As compared to the CoMFA method on the same compounds, the *k*NN program provided QSAR models with higher q^2 and predictive R^2. One of the best models was obtained from *k*NN analysis with topological indices as descriptors and provided q^2 and predictive R^2 values of 0.60 and 0.62, respectively, compared with only 0.41 and 0.36 for the CoMFA approach. The high predictive ability of the model allows for virtual screening of large databases and rational design of focused libraries. Novel 4β-amino analogues (**26**−**30**) were designed and synthesized based on the CoMFA and kNN QSAR models. Several of these analogues showed greater cytotoxicity and topo II inhibitory potency than etoposide and were not cross-resistant to KB-7d cells. Most of these analogues showed preclinical activity superior to that of GL331, in terms of DNA break induction and cell killing.[44] The success of these modeling studies may improve the further design of novel biologically active epipodophyllotoxin derivatives.

Other Plant-Derived Antineoplastic Agents and Their Analogues (Figures 4, 5)

In continuing bioactivity-directed fractionation and isolation of medicinal herbs (primarily herbs of Chinese origin), many cytotoxic lead compounds have been discovered in the author's laboratory. Prior reviews have covered many of these compounds;[45,46] herein, selected natural products and their synthetic analogues, including recent research results, are described.

Brucea javanica (L.) Merr. (Simaroubaceae) ("Ya Tan Tzu") is the natural source of the cytotoxic quassinoids called bruceosides,[47−49] including bruceoside C (**31**) (ED_{50} < 0.1 μg/mL in KB and RPMI-7951 cell lines) and brusatol (**32**). *Brucea antidysenterica* Mill. (Simaroubaceae) contains the related compound bruceantin (**33**),[50] which has been tested in Phase II clinical trials, but has not progressed to drug development. Recently, four fluorinated compounds (**34**−**37**) were designed to prevent oxidation of the C-15 side chain and circumvent deactivation. One compound (**34**) was approximately as active as **33** in the eight human cancer cell lines. It contains a 4,4,4-trifluoro-3-methyl-butanoyl ester at C-15.[51]

Dysoxylum cumingianum C. DC. (Meliaceae) ("Lan Yu Kong Mu") yielded the antileukemic cumindysosides A and B (**38**, **39**) and cumingianosides A−D (**40**−**43**) (14,18-cycloapoeuphane-type skeleton)[52] and P (**44**) and Q (**45**)[53] (apotirucallane skeleton). Acid treatment of cumingianosides A, C, and E and cumindysoside A produced four novel compounds (**46**−**49**),[54] which had log GI_{50} values ranging from −7.11 to −4.94 and were especially potent against leukemia and colon tumor cell lines.

Three new cytotoxic clerodane diterpenes, bucidarasins A−C (**50**−**52**), were isolated from *Bucida buceras* L. (Combretaceae).[55] Their structures were elucidated from detailed 2D NMR analysis. Compounds **50**−**52** showed potent cytotoxicity against human tumor cell lines with IC_{50} values of 0.5−1.9 μM, which was retained in drug-resistant cell lines. A related compound without the acetal moiety was inactive.

Desmosdumotin C (**53**), a novel compound isolated from the roots of *Desmos dumosus* Roxb. (Annonaceae), showed significant and selective in vitro cytotoxicity against bone (HOS), breast (MCF), and ovarian (IA9) cancer cell lines.[56]

Garuga pinnata Roxb. (Burseraceae) is the natural source of pheophorbides-*a* (**54**) and -*b* methyl esters, which show broad photo-dependent cytotoxic activity. Compound **54** has ED_{50} = 0.6 μM in the light, but is completely inactive under dark conditions. In an SAR study of known and novel pheophorbide-*a* derivatives as photo-dependent and photo-independent cytotoxic agents, zinc chlorin-*e*6 trimethyl ester (**55**) possessed photo-independent cytotoxic activity, with ED_{50} = 2.4 μM against KB cells incubated in the light and 4.6 μM in the dark.[57] Thus, such zinc-chelated analogues may overcome a clinical deficiency of pheophorbide-based photosensitizers, their dependence on light penetration to the target tissue.

Three pheophorbide-related compounds (**56**−**58**) were isolated from the leaves and stems of *Clerodendrum calamitosum* L. (Verbenaceae).[58] Compounds **56** and **57** exhibited strong cytotoxicity against human lung carcinoma (A549), ileocecal carcinoma (HCT-8), kidney carcinoma (CAKI-1), breast adenocarcinoma (MCF-7), malignant melanoma (SK-MEL-2), ovarian carcinoma (1A9), and epidermoid carcinoma of the nasopharynx (KB), and its etoposide- (KB-7d), vincristine- (KB-VCR), and camptothecin-resistant (KB-CPT) subclones. Compound **58** was less cytotoxic than **56** and **57**; however, its methyl ester **59**, which was isolated from leaves of the related plant *Clerodendrum cyrtophyllum* Turcz (Verbenaceae), was more active than the methyl esters of **56** and **57**.

Two antileukemic natural flavonoids, tricin (**60**) and kaempferol 3-*O*-β-D-glucopyranoside (**61**), and one lignan, wilstromol (**62**), were isolated from *Wikstroemia indica* (L.) C. A. Meyer (Thymelaeaceae) ("Nan Ling Jao Hua") and showed %T/C values of 130−174% at 12.5−16 mg/kg in P388-infected mice.[59] Synthetic 2-phenyl-4-quinolones (e.g., **63**, **64**), which contain a ring nitrogen instead of the oxygen found in the natural compounds, also showed impressive differential cytotoxicity against human tumor cell lines and were potent inhibitors of tubulin polymerization.[60,61] One fluorinated compound (**63**, NSC 656158) demonstrated a 130% increase in life span when tested by NCI in the xenograft ovarian OVCAR-3 model.[62] The most potent compound thus far, 2-(2′-fluorophenyl)-6-pyrrolinyl-4-quinolone (**64**), had GI_{50} values in the nanomolar or subnanomolar range (average log GI_{50} = −7.65 and ≤−8.00 in renal and melanoma cell lines).[63]

In a series of 1,2,3,4-tetrahydro-2-phenyl-4-quinolones (THPQ) (e.g., **65**, **66**), which are hydrogenated rather than having a double bond in the quinolone B ring, the most active compound, 2,3-dihydro-2-(3′-methoxyphenyl)-6-pyrrolinyl-4-(1*H*)-quinolone (**65**), had ED_{50} values ranging from 0.008 to 0.11 μg/mL in six tumor cell types.[64] Stereospecificity was found with the (−)-enantiomer of **66** being ca. 5-fold more active than the (+)-enantiomers in inhibiting tubulin polymerization.[64]

Another structurally related series, the 2-aryl-1,8-naphthyridin-4-ones (e.g., **67**−**70**), contain a second nitrogen at position 8 in the aromatic A ring. Analogues with methyl groups at C-6 or C-7 and with *meta*-substituted phenyls (methoxy, chloro, or fluoro) at C-2 were quite potent in NCI's 60 human tumor cell line panel with GI_{50} values ranging from −6.57 to −7.72 and log GI_{100} from −4.14 to −6.49.[65,66]

The alkaloid colchicine (**71**) (Figure 5) is isolated from *Colchicum autumnale* L. (Liliaceae) and has long been and is still used medicinally, currently to treat gout and familial Mediterranean fever. Colchicine and its synthetic analogue, thiocolchicine (**72**) (SCH_3 rather than OCH_3 at C-10), are mitotic inhibitors, which inhibit polymerization of tubulin (ITP), with IC_{50} values of 1.5 and 0.65 μM, respectively.[67] Although they show antileukemic activity, they are too toxic to use as anticancer agents. Thus, the NPL has synthesized many new analogues that might overcome this deficiency. The C-7 acetamido group on the B ring has been a target of modification and has been replaced by ketone (**73**, thiocolchicone), hydroxy (**74**), and ester (**75**, **76**) groups.[68] Thiocolchicone and the (−)-*aS*,7*S* optically pure enantiomers [the C-7 alcohol, (−)-**74**, and its acetate, (−)-**75**, and isonicotinoate, (−)-**76**, esters] were equally or more active (ITP IC_{50} values ranging from 0.56 to 0.76 μM) than thiocolchicine. In a C-ring modification, deaminodeoxycolchinol-7-one thiomethyl ether or allo-ketone (**77**) was synthesized by reacting thiocolchicone (**72**) with aniline, causing ring contraction and forming a six-membered C ring rather than the seven-membered ring found in the natural product.[69] This analogue was equipotent with its seven-membered-ring parent compound. Three related ring-contracted colchicinoids (**78**−**80**), with OCH_3 rather than SCH_3 substitution on the C ring, showed excellent activities in drug-sensitive and -resistant KB cell lines.[69] Investigation of demethylated A-ring colchicine analogues

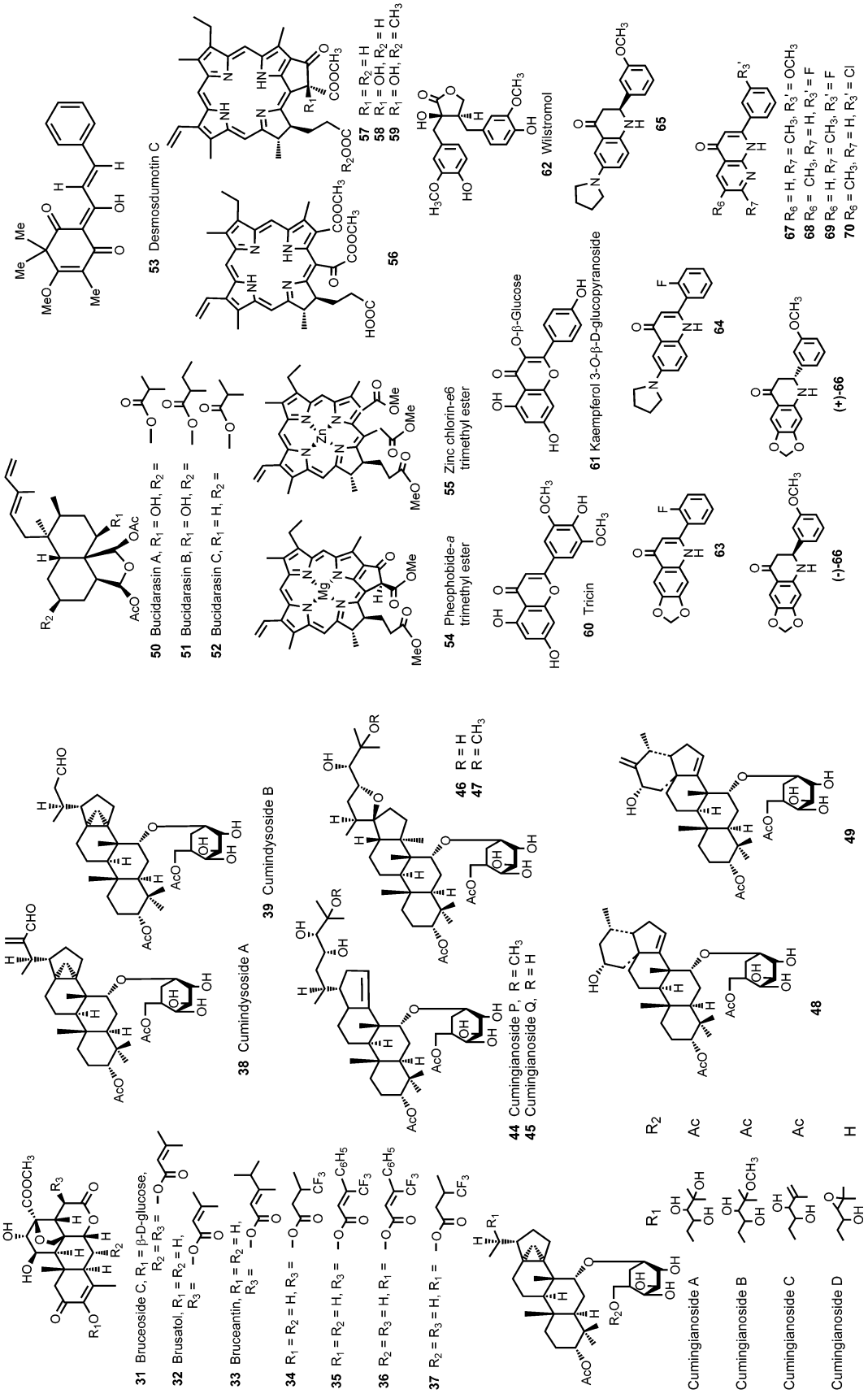

Figure 4. Other plant-derived antineoplastic compounds and analogues.

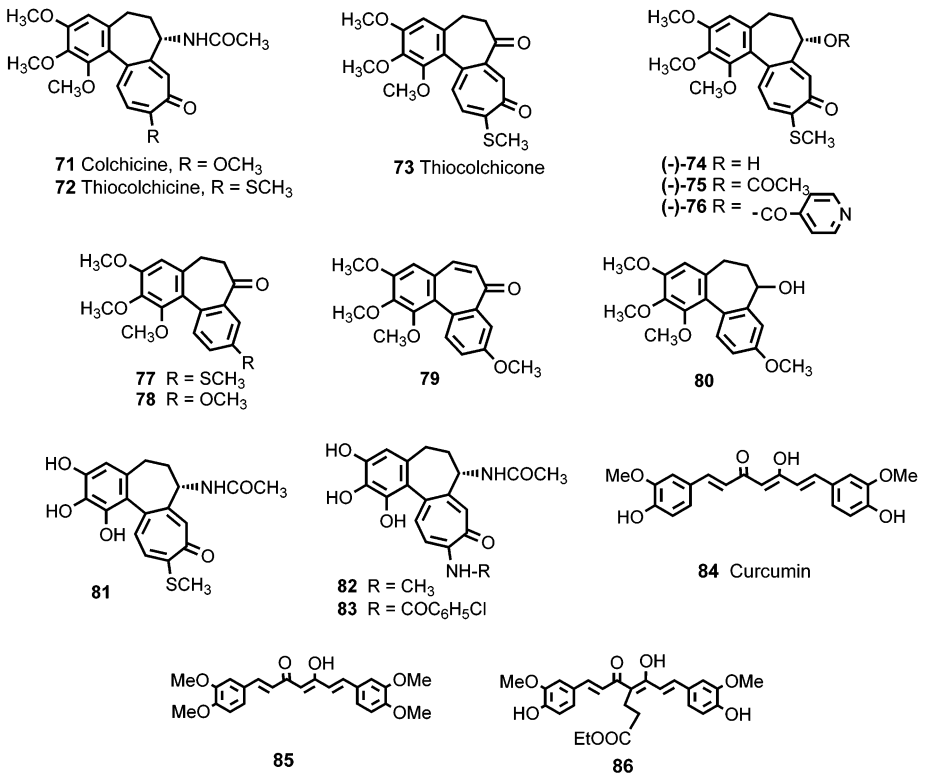

Figure 5. Colchicine and curcumin analogues.

Table 1. U.S.-FDA-Approved AIDS Drugs

NRTIs	NNRTIs	PIs	FIs
zidovudine (3′-azido-3′-deoxythymidine, AZT, Retrovir)	nevirapine (Viramune)	saquinavir (Inverase or Fortovase)	enfuvirtide (Fuzeon)
zalcitabine (dideoxycytidine, ddC, Hivid)	delavirdine (Rescriptor)	ritonavir (Norvir)	
didanosine (dideoxyinosine, ddI, Videx)	efavirenz (Sustiva)	indinavir (Crixivan)	
stavudine (2′,3′-didehydro-3′-deoxythymidine, d4T, Zerit)		nelfinavir (Viracept)	
lamivudine (2′,3′-dideoxy-3′-thiacytidine, 3TC, Epivir)		amprenavir (Agenerase)	
abacavir (Ziagen)		atazanavir (Reyataz)	
tenofovir (Viread)		Kaletra = combination of ritonair and lopinavir	
emtricitabine (Emtriva)			
Combivir and Trizivir = combinations of the above NRTIs			

led to a very interesting result. Full tubulin binding affinity and inhibition of tubulin polymerization (ITP) requires all three phenolic groups in the A ring to be methylated. However, in a topo II inhibition assay, 1,2,3-demethylthiocolchicine (**81**) was more potent than etoposide.[70] Thus, tridemethylated colchicines and thiocolchicines are a new class of DNA topo II inhibitory agents. Two compounds (**82**, **83**) with an acetamido group at C-7 and amino or amido groups at C-10 showed topo II inhibitory and cytotoxic activity with ED_{50} values of 0.36 and 0.48 μg/mL against the MCF-7 breast cancer cell lines and 0.72 and 0.98 μg/mL against the CAKI-1 kidney cancer cell line.[71]

Curcumin, a phenolic diarylheptanoid (**84**), is the major pigment in turmeric. Curcumin and its analogues show various biological activities, including cytotoxicity.[72] Several synthetic curcumin analogues, including **85** and **86**, showed potent antiandrogenic activities against two human prostate cancer cell lines, PC-3 and DU-145, and were superior to hydroxyflutamide, which is the currently available antiandrogen for the treatment of prostate cancer.[73] This new class of antiandrogen agents could be developed into clinical trial candidates to control androgen receptor-mediated prostate cancer growth.

Plant-Derived Anti-HIV Agents and Their Analogues (Figures 6, 7)

In addition to the anticancer drug development research, the NPL also has a vigorous program to discover and develop plant-derived compounds for anti-AIDS therapies.

Although significant treatment advances have been made, acquired immunodeficiency syndrome (AIDS), a degenerative disease of the immune and central nervous systems, continues to be an enormous, incurable health threat and, particularly in less developed countries, to have a high fatality rate. The human immunodeficiency virus (HIV) is the causative agent and can potentially be attacked chemotherapeutically at several points in its life cycle: virus adsorption, virus−cell fusion, uncoating, HIV regulatory proteins, HIV enzymes (reverse transcriptase, integrase, and protease), and virus budding/maturation.

Treatment options have expanded during the past few years, with 19 drugs now approved for clinical use in the United States (Table 1). These drugs fall into four general categories: nucleoside/nucleotide viral reverse transcriptase (RT) inhibitors (NRTIs), non-nucleoside RT inhibitors (NNRTIs), protease inhibitors (PIs), and fusion (or entry) inhibitors (FIs).[74]

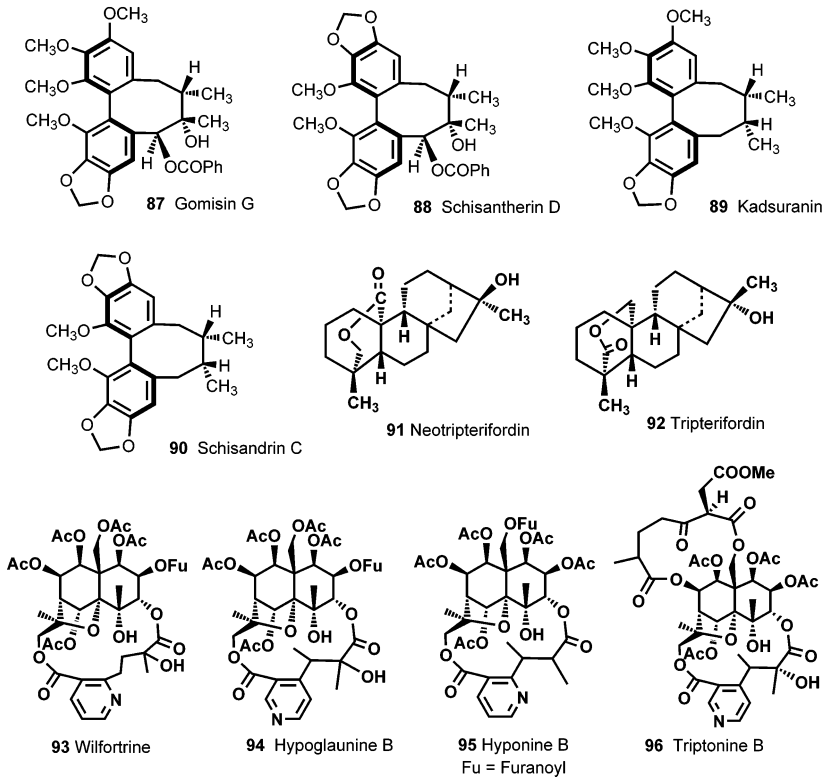

Figure 6. Anti-HIV lignan and diterpene compounds.

3′-Azido-3′-deoxythymidine (AZT) was the first AIDS drug developed and approved. It is effective in increasing the survival time of AIDS patients and in reducing the severity and frequency of opportunistic infections, which plague infected individuals. However, the effectiveness of AZT and other NRTIs is compromised by their severe adverse side effects, including marrow suppression, anemia, and peripheral neuropathy. Most importantly, mono- and bi-therapy for HIV type 1 (HIV-1) infection are only transiently effective due to the development of viral resistance;[75−77] thus, at least triple-drug combinations are currently recommended.[78−80] Triple-drug cocktails, e.g., often containing one PI and two NRTIs (ddC and 3TC), can reduce levels of viral RNA to undetectable levels in the plasma and slow the development of viral resistance. However, many of the drugs cause severe toxicities, require complicated dosing schedules that are hard to maintain, and are quite costly, which especially limits their use in underdeveloped countries where infection is most prevalent. The new FI, Fuzeon, has been approved only for patients who have failed other drug regimens, not for drug-naïve patients, and is quite difficult to make.

Therefore, the NPL and other laboratories are continuing anti-HIV drug development needed to answer the issues mentioned above. Ideally, new drugs will have structures and mechanism of action distinct from those of current drugs and also show minimal toxicity, be manufactured at lower cost, and be suitable for combination therapy. The NPL continues both to screen plant extracts, particularly anti-infective or immunomodulating Chinese herbal medicines, and conduct extensive structural modification of discovered leads.

Lignans (Figure 6). Among known lignans isolated from *Kadsura interior* A. C. Smith (Schizandraceae) ("Ji Xue Teng"), gomisin G (**87**) has been found to be the most potent (EC_{50} = 0.006 μg/mL; TI = 600) inhibitor of HIV replication. Schisantherin D (**88**), kadsuranin (**89**), and schisandrin C (**90**) were also quite active: the respective EC_{50} and TI values are 0.5, 0.8, and 1.2 μg/mL and 110, 56, and 33.3. In the cyclooctane ring, the position and substitution of hydroxy groups were important to enhanced anti-HIV activity.[81]

Diterpenes (Figure 6). *Tripterygium wilfordii* Hook. (Celastraceae) ("Lai Gong Teng"), a poisonous liana found in southern mainland China, yielded a new kaurane-type diterpene lactone, neotripterifordin (**91**).[82] It inhibited HIV replication in H9 lymphocytes with an EC_{50} of 25 nM and a TI of 125, while the isomeric tripterifordin (**92**) was much less active, with EC_{50} and TI values of 5 μM (1 μg/mL) and 5, respectively.[82,83] This plant also contained three anti-HIV sesquiterpene alkaloids, wilfortrine (**93**), hypoglaunine B (**94**), and hyponine B (**95**), and a similar compound, triptonine B (**96**), was found in a related species, *T. hypoglaucum*. These four alkaloids showed EC_{50} values of <0.1 μg/mL and TI > 1000.[84]

Triterpenes (Figure 7). Betulinic (**97**) and platanic (**98**) acids from *Syzigium claviflorum* Wall. ex A. J. Cowan (Myrtaceae) ("Pang Hua Chih Nan") were identified as anti-HIV principles with EC_{50} values of 1.4 and 6.5 μM and TIs of 9.3 and 13.[85] Modification of betulinic and dihydrobetulinic acids has successfully increased their anti-HIV potency. Esterification at the C-3 hydroxyl resulted in potent compounds with tremendously improved TI values. 3-*O*-(3′,3′-Dimethylsuccinyl)betulinic acid (**99**, DSB, PA-457) had an $EC_{50} < 3.5 \times 10^{-4}$ μM and TI > 20 000; the latter value is higher than that of AZT (TI = 12 500) in the same assay.[86] This compound is currently in preclinical development and will be discussed in more detail later in this review. The same esterification coupled with bond saturation of the structurally related oleanolic acid (**100**) resulted in similar, though not quite as dramatic, results: **100**: EC_{50} = 1.7 μg/mL, TI = 12.8; **101**: EC_{50} = 0.0039 μg/mL, TI = 3,750.[87] DSB and other new leads were highlighted in a recent review of plant-derived terpenoids and analogues with respect to their anti-HIV activity, SAR, and mechanism of action.[88]

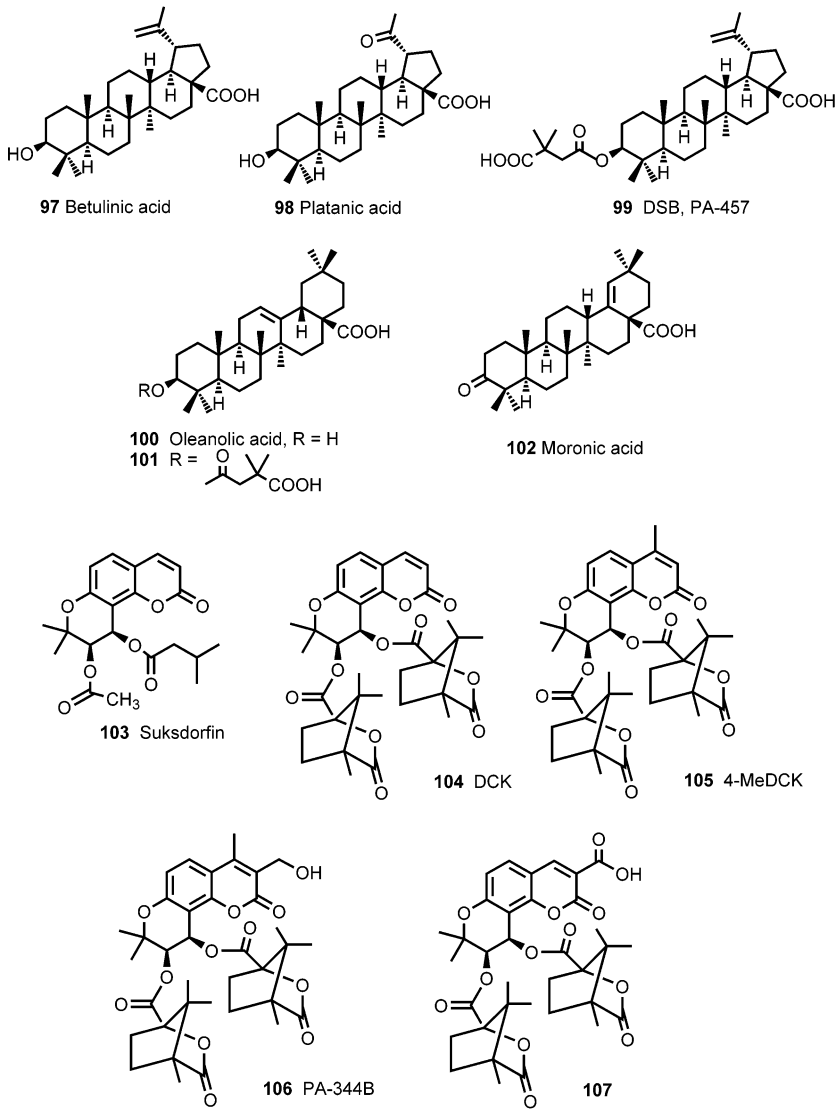

Figure 7. Anti-HIV triterpene and coumarin compounds.

Another known triterpenoid, moronic acid (**102**), isolated from Brazilian propolis, showed significant anti-HIV activity (EC_{50} < 0.1 μg/mL, TI > 186).[89] SAR studies are under investigation.

Coumarins (Figure 7). The pyranocoumarin suksdorfin [**103**, (3′*R*,4′*R*)-3′-acetoxy-4′-(isovaleryloxy)-3′,4′-dihydroselin] was isolated as anti-HIV principle (EC_{50} 1.3 μM, TI = 140) from *Lomatium suksdorfii* (Coult. & Rose) (Umbelliferae) ("Bei Mei Chian Hu") and *Angelica morii* ("Shan Du Huo").[90] Modification with different 3′,4′-esters led to 3′R,4′R-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (**104**) as an improved lead (DCK; EC_{50} 0.0004 μM, TI 136 719).[91] All four diastereoisomers were prepared to investigate the effect of stereochemistry, and the (*S*,*S*), (*R*,*S*), and (*S*,*R*) isomers were at least 10 000 times less active than the (*R*,*R*) isomer.[92] The activity of DCK was confirmed in several in vitro assays in different cell lines, including a clinical HIV-1 isolate in peripheral blood mononuclear cells.[91] In modification studies of the coumarin skeleton, adding methoxy or methyl groups at positions 3, 4, and 5, but not 6, resulted in retention or improvement of activity.[93,94] These studies led first to the more potent 4-MeDCK (**105**) (EC_{50} 1.6 × 10^{-7} μM, TI > 10^9) and then recently to the clinical candidate 3-hydroxymethyl-4-methylDCK (**106**, PA-344B) (more details below).[95] In order potentially to accelerate the synthesis and development of new DCK analogues, the NPL developed a new, straightforward, easily automated parallel solid-phase procedure to prepare 3′*R*,4′*R*-di-*O*-*cis*-acyl 3-carboxyl khellactones (**107**).[96]

Mechanism of Action and Preclinical Studies with DCK and DSB Analogues. The NPL's lead coumarin compound, PA-344B (**105**), is a nanomolar inhibitor of both primary clinical and drug-resistant HIV-1 isolates, but has no effect on HIV-2 or SIV. Compared to approved HIV drugs, it has a novel mechanism of action, as it blocks RT, but at a later step than the FDA-approved RT inhibitors. Mechanism studies are continuing. Disposition and pharmacokinetic studies support further development. PA-344B is orally bioavailable in rats and dogs with a plasma half-life of 2−3 h in rats (which may be longer in humans based on comparative in vitro metabolism data). In preclinical toxicology studies, minimal toxicities were found, and kilogram quantities have been manufactured. Panacos Pharmaceuticals, Inc. is the licensing company and has nearly completed the required preclinical studies for IND filing.[97]

The triterpenoid 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid (**99**, DSB, PA-457) has also been licensed by Panacos Pharmaceuticals for preclinical development. DSB has no effect on HIV RT, protease, or several other targets including entry. Studies at Panacos confirm a unique effect on viral budding/maturation.[98,99] DSB, thus, has a com-

pletely novel mechanism of action to the current AIDS drugs and is a principal clinical trials candidate. In rats, DSB is orally bioavailable with a half-life of 2−3 h. Results of ongoing preclinical safety tests are very promising. Additional studies (in vitro metabolism, protein binding, p*K* in dogs, GLP toxicology) are in progress preparatory to IND filing later in 2003.[97]

Conclusions

Chinese herbal medicine has a history of over 4000 years, dating back to its founder, the legendary emperor Shen Nung (2696 BC), who is credited with the classic work "Shen Nung Pen Tsao Ching" (The Book of Herbs by Shen Nung, compiled during the Han dynastry). Shen Nung grouped 365 herbs into three classes, upper, middle, and lower, based on herbal toxicities and their corresponding periods of use. Nontoxic and rejuvenating upper class herbs can be taken continuously for a long period, particularly as dietary supplements; middle class herbs can have either nontoxic or toxic effects and must be used with more caution; lower class herbs have toxic properties, cannot be taken for extended periods, and must be properly processed to reduce their toxicity before being used. The 365 herbs of this herbal classic and numerous historical and modern publications are still in use today as part of the traditional Chinese healing arts. Herbs from all classes can treat chronic and acute illnesses, while upper class herbs are ideal as dietary supplements. The folkloric uses of Chinese herbal medicine, from both oral and written tradition, have led to many instances of corresponding successful drug development, and the continuing discovery and development of bioactive natural products and their analogues as therapeutic agents are the goals of the NPL. For example, *Podophyllum emodi* (Kuei Chiu) is listed in the lower class (i.e., more toxic) drugs of "Shen Nung Pen Tsao Ching" and is used traditionally as a contact cathartic. Accordingly, podophyllotoxin was isolated as the cytotoxic principle from this herb. In the NPL, modification of podophyllotoxin subsequently led to the discovery of GL-331 (**20**), which is an analogue of etoposide and teniposide, two clinically useful anticancer drugs, and has now completed Phase I and is in planning for Phase II clinical trials as an anticancer drug.

Based on this and other above examples, new anticancer and anti-HIV drugs derived from research on plant-derived agents will be discovered continuously in the future. The natural products leads first identified can be further developed or modified to yield useful drugs or be subjected to biochemical and pharmacological studies to increase knowledge of tumor cell and viral biology. The discovery of new anticancer and anti-HIV drugs should be made easier as bioassay technology improves.

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References and Notes

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