Invited Review

Discovery and Development of Natural Product-Derived Chemotherapeutic Agents Based on a Medicinal Chemistry Approach[⊥,†]

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Medicinal plants have long been an excellent source of pharmaceutical agents. Accordingly, the long-term objectives of the author's research program are to discover and design new chemotherapeutic agents based on plant-derived compound leads by using a medicinal chemistry approach, which is a combination of chemistry and biology. Different examples of promising bioactive natural products and their synthetic analogues, including sesquiterpene lactones, quassinoids, naphthoquinones, phenylquinolones, dithiophenediones, neo-tanshinlactone, tylophorine, suksdorfin, DCK, and DCP, will be presented with respect to their discovery and preclinical development as potential clinical trial candidates. Research approaches include bioactivity- or mechanism of action-directed isolation and characterization of active compounds, rational drug design-based modification and analogue synthesis, and structure−activity relationship and mechanism of action studies. Current clinical trial agents discovered by the Natural Products Research Laboratories, University of North Carolina, include bevirimat (dimethyl succinyl betulinic acid), which is now in phase IIb trials for treating AIDS. Bevirimat is also the first in a new class of HIV drug candidates called "maturation inhibitors". In addition, an etoposide analogue, GL-331, progressed to anticancer phase II clinical trials, and the curcumin analogue JC-9 is in phase II clinical trials for treating acne and in development for trials against prostate cancer. The discovery and development of these clinical trial candidates will also be discussed.

Introduction

In the Natural Products Research Laboratories (NRPL), our objectives are to discover and develop bioactive natural products and their analogues as clinical trial candidates. The three approaches used to achieve these objectives are (1) bioactivity- or mechanism of action-directed isolation and characterization of active compounds, (2) rational drug design-based modification and analogue synthesis, and (3) mechanism of action (MOA) studies. The scientific disciplines covered include natural products chemistry, molecular biology and biochemistry, and pharmacology, to discover promising new leads based on bioactivity- or mechanism of action-directed approaches; medicinal chemistry and synthetic organic chemistry to achieve new leads optimization based on modern medicinal chemistry approaches; and analytical chemistry to apply state-of-the-art analytical instrumental chromatography technologies to support the above two tasks. MOA and in vivo evaluation studies are supported by collaborations with more than 60 active established researchers worldwide to enhance the programs of the NPRL. Current research programs in the NPRL include the investigation of (1) novel plant cytotoxic antitumor and anti-HIV principles and synthetic analogues as antitumor and anti-AIDS agents and (2) other chemotherapeutic agents, such as antimalarial, antifungal, antiviral, and anti-inflammatory agents, as well as (3) traditional Chinese medicines (TCM), targeting their active principles, fractions, and prescriptions.

General Concepts on Drug Discovery and Development

Drug discovery can build on several sources; however, my laboratories focus on bioactivity-directed isolation and characterization of lead natural product principles from single medicinal herbs and formulations. As shown in Figure 1, the subsequent preclinical optimization of a lead compound is a cyclical process of obtaining bioassay screening results, analyzing activity data, designing new target compounds, and synthesizing new analogues.[1] In this iterative process, I feel that chemistry and biology are complementary and codependent areas of science, similar to the Chinese concepts of Yin and Yang: one is not present or sufficient without the other (Figure 2). The discovery of new bioactive compounds depends on valid biological assays, while new chemistry can make the discovery of new biological targets possible. I feel that medicinal chemistry combines techniques from chemistry and from biology to facilitate new drug discovery. By using these concepts and techniques, the NPRL has been able to discover more than 3000 bioactive natural products and their synthetic derivatives/analogues since 1971, as briefly summarized below.

Antitumor Agents

Sesquiterpene Lactones. Helenalin and Its Analogues. In the mid-1970s, three sesquiterpene lactones, molephantin (**1**),[2] molephantinin (**2**),[3] and phantomolin (**3**) (Figure 3),[4] were discovered as new cytotoxic principles from *Elephantopus mollis*. The latter two compounds showed similar T/C values (397% and 378%, respectively) against Walker 256 carcinoma (W-256) in rats (dose = 2.5 mg/kg). Microlenin (**4**), a structurally related dimeric sesquiterpene lactone from *Helenium microcephalum*, had a T/C value of 173% against W-256 (dose = 2.5 mg/kg),[5] while its monomer, helenalin (**5**), had a T/C value of 316% under the same conditions (Figure 4). Helenalin contains both an α-methylene-γ-lactone moiety and an α,β-unsaturated ketone; therefore, studies were performed to determine the relative contributions of the two O=C−C=CH_2 systems. The rank order of potency against Hep-2 human epidermoid laryngeal carcinoma was **5** (ED_{50} 0.08 μg/mL) > 11,13-dihydrohelenalin (**6**) (ED_{50} 0.80 μg/mL) > 2,3-dihydrohelenalin (**7**) (ED_{50} 3.84 μg/mL) > 2,3,11,13-tetrahydrohelenalin (**8**) (inactive) (Figure 5).[6] Thus, reduction of the double bond of the α,β-unsaturated ketone had a greater effect on potency than reduction of the α-methylene-γ-lactone. In mechanistic studies, the cytotoxicity of helenalin and structurally related sequiterpene lactones was linked to a Michael-type addition reaction of the

[⊥] Dedicated to the late Dr. John W. Daly of NIDDK, NIH, Bethesda, Maryland, and to the late Dr. Richard E. Moore of the University of Hawaii at Manoa for their pioneering work on bioactive natural products.

[†] Antitumor Agents 275 and Anti-AIDS Agents 80. Adapted from a Norman R. Farnsworth Research Achievement Award address presented at the 50th Annual Meeting of the American Society for Pharmacognosy, Honolulu, HI, June 27−July 1, 2009.

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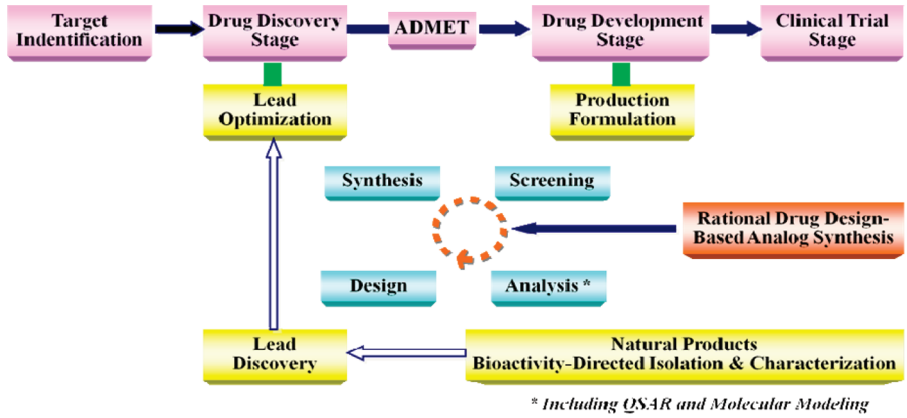


Figure 1. Flowchart for drug discovery and development of natural products-derived chemotherapeutic agents. From: Qian, K.; Nitz, T. J.; Yu, D.; Allaway, G. P.; Morris-Natschke, S. L.; Lee, K. H. In *Natural Product Chemistry for Drug Discovery*; Buss, A. D., Butler, M. S., Eds., RSC Publishing: Cambridge, UK, 2010; p 376. Reproduced by permission of The Royal Society of Chemistry.

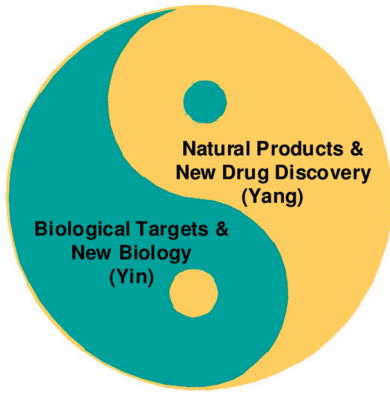


Figure 2. Complementarity of chemistry and biology: medicinal chemistry is an art of combining chemistry and biology for drug discovery.

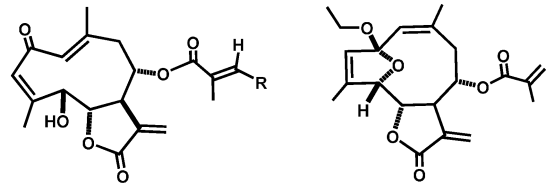


Figure 3. Structures of cytotoxic natural sesquiterpene lactones from *Elephantopus mollis*.

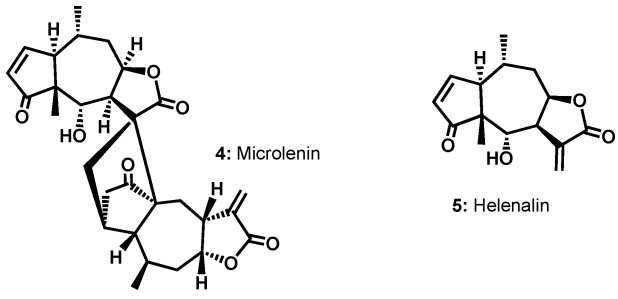


Figure 4. Structures of cytotoxic natural pseudoguaianolides from *Helenium microcephalum*.

O=C−C=CH_2 systems in the molecule with sulfhydryl groups of reduced glutathione and L-cysteine.[7,8] Finally, bis(helenalinyl) esters (two helenalin molecules connected through their hydroxy group by a diester linkage) were generally more potent and less toxic than the parent alcohol.[9] At 8 mg/kg, **5** had a T/C of 162% against P388 leukemia in mice, while bis(helenalinyl)glutarate (**9**, Figure 6) had a T/C of 195%.[9]

Quassinoids: Brusatol and Its Analogues. The fruits of *Brucea javanica* (Chinese medicine "Ya-Tan-Tzu") yielded many new quassinoids, including several compounds with significant cytotoxicity against various cancers, such as bruceosides A−F (**10**−**15**) and brusatol (**16**) (Figure 7).[10−12] Bruceantin (**17**), which has a terminal isopropyl rather than methyl group in the C-15 ester side chain compared with **16** (Figure 7), was previously isolated from *B. antidysenterica* by Kupchan et al. by bioactivity-guided fractionation.[13] Our laboratories first reported two synthetic methods for the conversion of **10** into **17**, which was in anticancer clinical trials.[14] Connecting two molecules of **16** or **17** at the C-3 hydroxy group through malonate, glutarate, adipate, and sebacate esters gave bis-esters (**18**−**23**, Figure 8) that were as active or more active than the parent alcohols against P-388 leukemia.[15] In addition to C-3 esterification, other structural features essential for enhanced cytotoxic activity include free hydroxy groups at C-11 and -12, an enone double bond in ring A, and an unsaturated ester at C-15.[16,17] The identity of the C-15 ester side chain can significantly affect cytotoxicity, and oxidation of the C-15 side chain has been postulated to cause deactivation of **16**- or **17**-related quassinoids. Therefore, trifluoromethyl groups were incorporated into the side chain at this position, as well as in the C-3 ester side chain. The most potent analogue was 15-[3′-(trifluoromethyl)butanoyl]bruceolide (**24**, Figure 8), which had similar potency and log GI_{50} values (−7.0 to −8.7) compared with **17** against a human cancer cell line panel.[18]

Phenylquinolones and Naphthyridinones: NSC-656158 and Its Analogues. The natural flavonoids tricin (**25**) and kaempferol-3-*O*-*β*-D-glucopyranoside (**26**) and the lignan (+)-nortrachelogenin (**27**) were isolated by bioactivity-guided fractionation as antileukemic principles from *Wikstroemia indica* (Figure 9).[19] 2-Phenyl-4-quinolones are structurally related compounds with nitrogen rather than oxygen in the heterocyclic ring. Synthetic modification led to several fluorinated 2-phenyl-4-quionolones as potent antimitotic antitumor agents. NSC-656158 (**28**, Figure 10) showed potent cytotoxicity against a human tumor cell line (HTCL) panel (average log GI_{50} −6.47), inhibited tubulin polymerization (IC_{50} 0.85 μM), and exhibited good in vivo antitumor activity (130% prolonged life span of mice bearing OVCAR-3 xenografts).[20] An analogue (**29**, Figure 10) substituted with a pyrolidine ring rather than methylenedioxy group was even more potent, with an average in vitro log GI_{50} of −7.65 and tubulin inhibitory IC_{50} of 0.46 μM.[20] Mechanistically, **28** inhibits hepatocyte growth factor-induced invasion of SK-Hep-1 human hepatocellular carcinoma cells by suppressing matrix metalloproteinase-9 expression at the

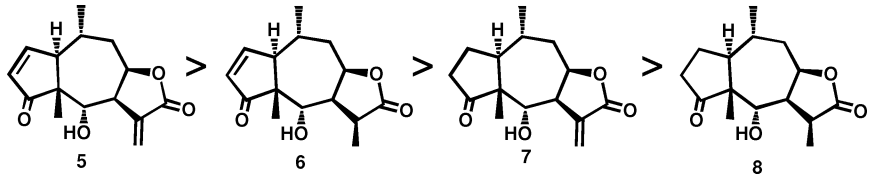


Figure 5. Rank order of cytotoxic potency of helenalin analogues with varying degrees of molecular unsaturation.

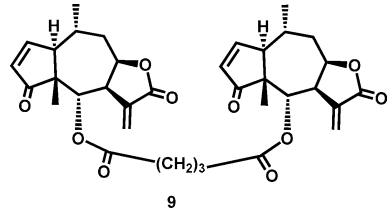


Figure 6. Structure of bis(helenalinyl)glutarate.

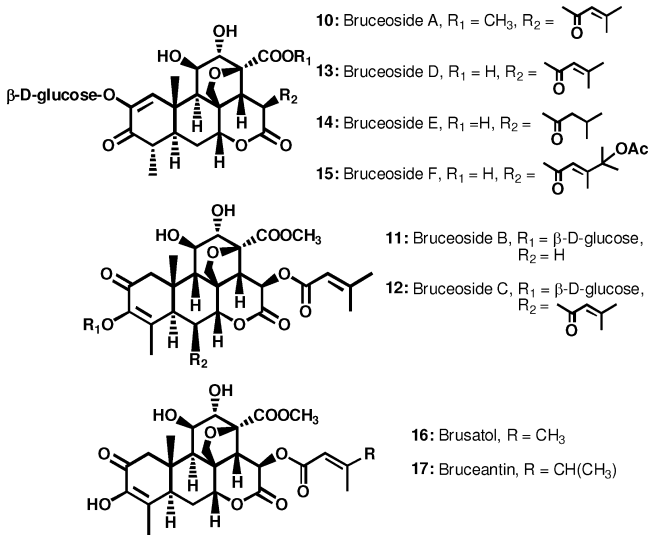


Figure 7. Structures of cytotoxic natural quassinoids from *Brucea* species.

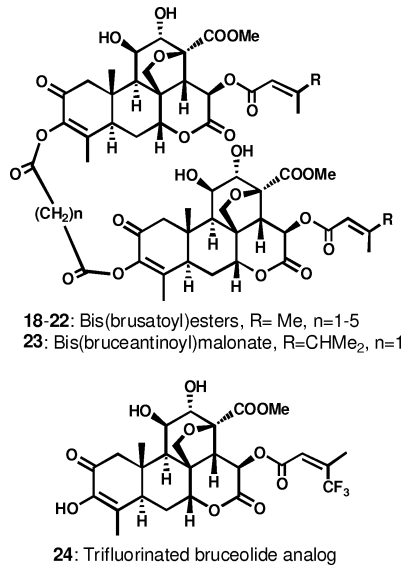


Figure 8. Structures of cytotoxic synthetic quassinoids.

micromolar range. Therefore, **28** is a potential therapeutic agent against tumor invasion.[21] 2-Aryl-1,8-naphthyridin-4(1*H*)-ones were also synthesized as antimitotic antitumor agents. 3′-Methoxy and halogenated-

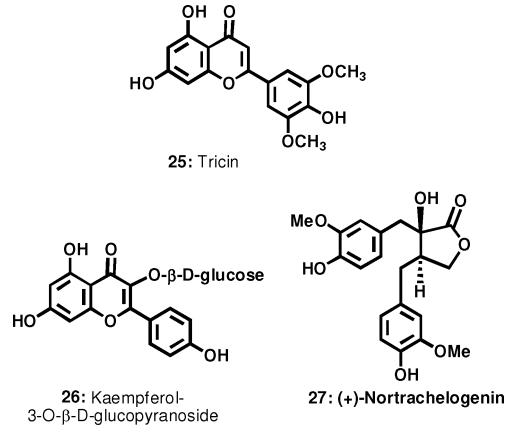


Figure 9. Structures of antileukemic natural flavonoids and lignan from *Wikstroemia indica*.

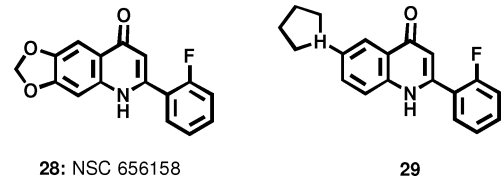
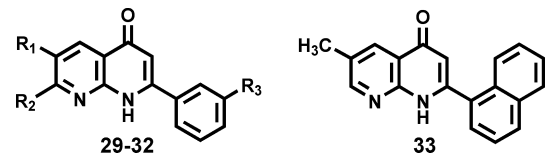


Figure 10. Structures of cytotoxic synthetic fluorinated 2-phenyl-4-quinolones.

Table 1. Antimitotic and Cytotoxic Activity of Synthetic Naphthyridinones



cmpd	R_1	R_2	R_3	ITP[a] IC_{50} (μM)	ICB[b] % inhibition	cytotoxicity log GI_{50}
29	H	Me	OMe	0.75	29	−7.24
30	Me	H	F	0.63	43	−7.30
31	H	Me	F	0.53	41	−7.37
32	Me	H	Cl	0.72	33	−6.57
33	see structure above			0.55	46	−7.72
positive control						
colchicine				0.80		−7.24
podophyllotoxin				0.46	76	−7.54

[a] ITP = inhibition of tubulin polymerization. [b] ICB = inhibition of colchicine binding.

2-phenyl compounds (**29**−**32**), as well as 2-(α-naphthyl) (**33**) but not 2-(*β*-naphthyl) compounds, were highly active in both antitumor and antitubulin assays, with average log GI_{50} values comparable to those of positive controls colchicine and podophyllotoxin (Table 1).[22] The identities of the halogens on the 2-phenyl ring and of substituents on the pyridine ring of the naphthyridinone system also influenced the tumor cell line selectivity at the total growth inhibition level.[22]

Naphthoquinones and Dithiophenedione Analogues. Psychorubrin (**34**, Figure 11) from *Psychotria rubra* is a cytotoxic

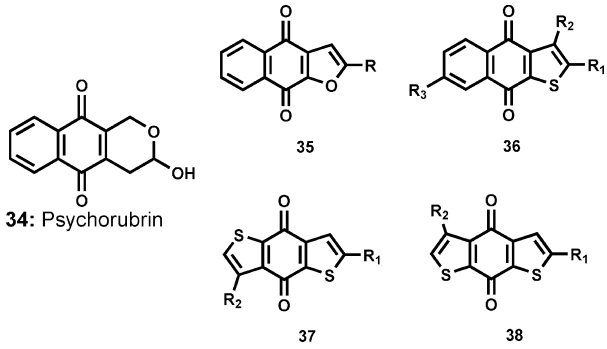


Figure 11. Structures of cytotoxic phychorubrin and other quinone analogues.

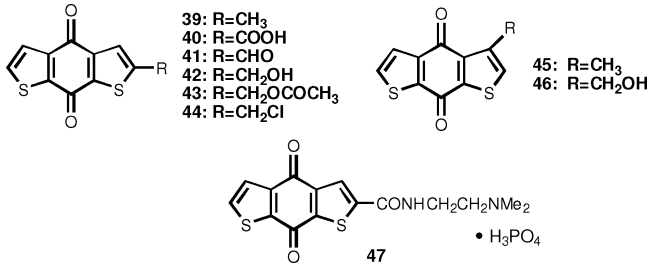
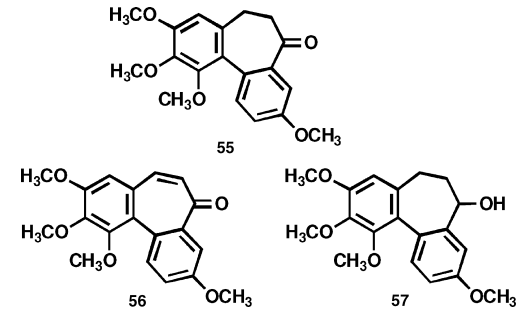


Figure 12. Structures of cytotoxic dithiophenedione analogues.

natural product with a naphthoquinone skeleton. Related compounds with furanonaphthoquinone (**35**) and naphthothiophenedione (**36**−**38**) skeletons (Figure 11) also show potent cytotoxicity.[23] Continued work led to a series of potent 2- and 3-methyl-4,8-dihydrobenzo[1,2-*b*:5.4-*b*′]dithiophene-4,8-diones (**39**−**46**, Figure 12). Several compounds showed high cytotoxic activity against melanoma, non small cell lung cancer, and breast cancer cell lines. 2-Hydroxymethyl-4,8-dihydrobenzo[1,2-*b*:5.4-*b*′]dithiophene-4,8-dione (**42**) showed the highest activity against melanoma (mean log $GI_{50} = -7.74$) and the highest overall potency (mean log $GI_{50} = -6.99$) against the NCI HTCL panel.[24] Dithiophene compounds were also found to significantly enhance retinoic acid-induced differentiation in leukemia cell lines. *N*-(2-Dimethylaminoethyl)-4,8-dihydrobenzo[1,2-*b*:5.4-*b*′]dithiophene-4,8-dione-2-carboxamide (**47**, Figure 12) showed the greatest effect, inducing nearly complete differentiation at 0.02 μM.[25]

Thiocolchicone Analogues. Both colchicine (**48**) and thiocolchicine (**49**) are potent antimitotic agents, inhibiting tubulin polymerization with IC_{50} values of 1.5 and 0.65 μM, respectively.[26]

Table 2. Cytotoxicity of Allocolchicinoids in Drug-Sensitive and Drug-Resistant KB Cell Lines



	cytotoxicity EC_{50} (μg/mL)				ITP IC_{50}
cmpd	KB[a]	KB-7d[b]	KB-VCR[c]	KB-CPT[d]	(μM ± SD)
55	0.057	0.025	>0.063 (42−69)	0.052	1.0 ± 0.1
56	0.029	0.024	0.016	0.042	1.0 ± 0.03
57	0.020	0.013	0.013	0.022	2.0 ± 0.2

[a] KB, epidermoid carcinoma of the nasopharynx. [b] KB-7d, KB cells with multidrug-resistant protein. [c] KB-VCR, KB cells with overexpression of *P*-glycoprotein. [d] KB-CPT, KB cells with reduced level of topoisomerase.

Thiocolchicone (**50**−**54**) derivatives, which have an oxygen moiety rather than a acetamido group at C-7, showed comparable or greater activity in tubulin polymerization, colchicine-binding, and cytotoxicity assays (Figure 13).[27] Allocolchinoids (**55**−**57**), with a six-membered rather than seven-membered C-ring, also showed significant antitubulin effects and cytotoxicity, even against three drug-resistant KB cell lines compared with the parental KB cell line (Table 2).[28]

Epipodophyllotoxins: GL331 and Its Analogues. Podophyllotoxin (**58**) is a natural lignan found in *Podophyllum peltatum* (or Mayapple) and related species. It inhibits mitosis by preventing polymerization of microtubules into tubulin. In contrast, etoposide (**59**) and teniposide (**60**), two semisynthetic glycosidic 4′-demethylepipodophyllotoxin derivatives (Figure 14),[29] do not affect tubulin, but instead act by inhibiting DNA topoisomerase II (topo II).[30] Although they are used clinically against various cancers, both compounds are poorly bioavailable, can cause myelosuppression, and suffer from drug resistance.[30] As a possible solution, 4-alkyl-,[31] benzyl-,[32] and aryl-[32] amino analogues of 4′-demethylepipodophyllotoxin were synthesized. Many compounds exhibited more potent DNA topo II inhibition and a greater percentage of protein-linked DNA breakage compared with etoposide.[32] When compared

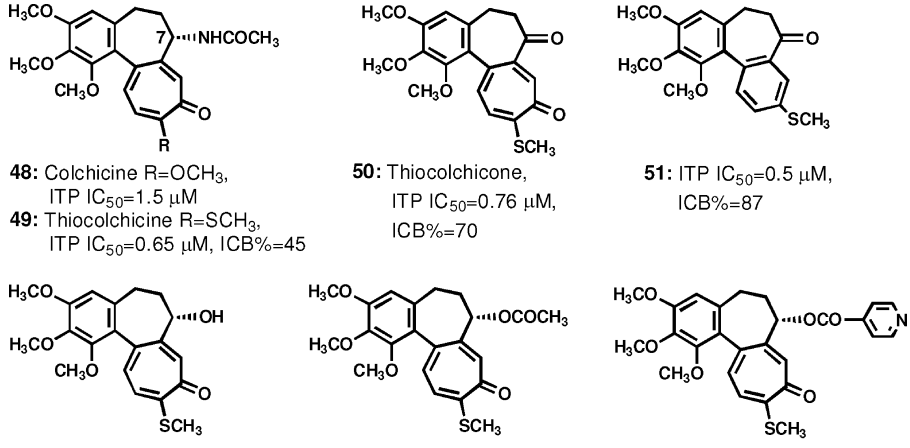


Figure 13. Structures and antimitotic activity of colchicine, thiocolchicine, and thiocolchicone analogues.

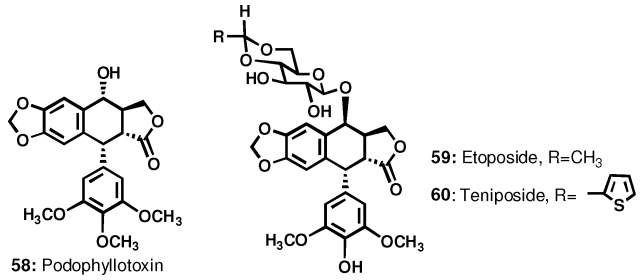


Figure 14. Structures of podophyllotoxin, etoposide, and teniposide.

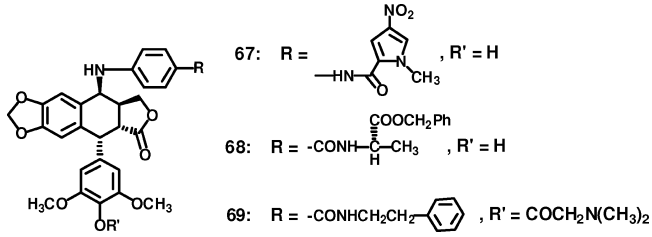
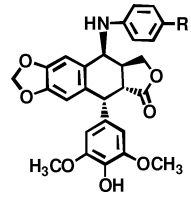


Figure 15. Structures of novel 4β-arylamino etoposide analogues.

Table 3. Cytotoxicity of Arylamino Analogues of Etoposide against KB and Drug-Resistant Sublines



		IC$_{50}$ (μM)			
cmpd	R	KB	KB1C	KB7D	KB50
61	NH_2 HCl	0.59	3.5	7.6	2.0
62	CN	0.61	2.7	5.0	4.0
63	**NO_2**	**0.49**	**6.1**	**7.7**	**3.0**
64	F	0.67	4.0	8.3	7.2
65	CO_2CH_3	0.84	2.5	7.0	3.3
66	$-OCH_2CH_2O-$	0.68	0.5	1.0	1.6
positive control etoposide		0.60	34.8	77.5	28.7

with etoposide, several arylamino compounds (**61**−**66**) were as cytotoxic against KB cells and more active against three KB cell lines (KB1C, KB7D, KB50) showing resistance to etoposide (Table 3).[32,33] GL331 [4′-*O*-demethyl-4β-(4″-nitroanilino)-4-deoxypodophyllotoxin] (**63**) emerged as the lead compound from these studies. Compound **63** (NSC-628679) was tested in phase I anticancer clinical trials at M. D. Anderson Cancer Center, after favorable toxicological evaluation by Genelabs Technologies, Inc. (Redwood City, CA). It showed markedly favorable results in these trials, with primary indications against colon and small cell lung cancers, and progressed to phase II clinical trials. Advantages of **63** over etoposide are easier manufacture, greater activity, particularly against drug-resistant cancer cell lines, and possibly a superior safety profile.[34] A comparative field analysis (CoMFA) computer model was generated using 102 epipodophyllotoxin, which showed that active compounds should have a positively charged functional group near the DNA minor groove.[35] Subsequent new analogues are shown in Figure 15. Adding bulky tails at the para position of the 4′-aniline resulted in improved activity profiles. Analogue **67**, with a pyrrolecarboxamidine moiety, displayed increased cytotoxicity (ED$_{50}$ = 0.04 and 0.2 μM) compared with etoposide (ED$_{50}$ = 0.2 and 25 μM) against both KB and KB-7d cells.[36] Similarly, compound **68**, with an amino acid (benzyl L-alanyl-*N*-carbonyl) incorporated at this same position, showed even better activity (ED$_{50}$ KB = 0.5 μg/mL, KB-7d = 0.25 μg/mL) than **63** (ED$_{50}$ = 0.2 μg/mL, 2 μg/mL) against drug-resistant cell lines.[37] Finally, the 4′-hydroxy group of 4β-para-substituted arylamino-epipodophyllotoxin analogues was esterified with *N*,*N*-dimethylglycine (**69**) to enhance drug resistance and water solubility simultaneously. Cytotoxicity and drug resistance profiles of most analogues were similar to those of **63**, while esterification caused some decrease in protein-linked DNA breaks and, thus, perhaps interaction with DNA.[38]

Curcumins: JC-9 (ASC-J9) and Its Analogues. The diarylheptanoid curcumin (**70**, Figure 16) is found in *Curcuma longa* and other related species. The rhizomes of these plants are used as both spices (turmeric) and medicines, particularly for hepatic disorders. Curcumin and other polyphenolic curcuminoids give turmeric its yellow color and stimulate bile secretion in the treatment of hepatitis. Curcumin itself shows moderate inhibitory activity against prostate cancer cell lines, and synthetic modifications yielded two lead compounds, JC-9 (**71**) and LL-80 (**72**), with increased activity against PC-3 (IC$_{50}$ = 1.1 and 1.0 μM, respectively) and LNCaP (IC$_{50}$ = 1.3 and 0.2 μM, respectively) cell lines.[39,40] Compound **71** (also known as ASC-J9) was licensed by Androscience Corp. (San Diego, CA) and has succeeded in phase II clinical trials against acne. Antiprostate clinical trials with **71** are being planned. Mechanistically, **71** enhances androgen receptor degradation.[41–43]

Neo-tanshinlactone Analogues. The rhizomes of *Salvia miltiorrhiza* are well known in TCM as "Tanshen" and used mainly to treat coronary disorders, such an angina pectoris. Among its lipophilic bioactive constituents are the tanshinones, including tanshinones I (**73**) and IIA (**74**) (Figure 17). The water-soluble sulfonate (**75**) of the latter compound may act as a calcium antagonist and anti-calmodulin drug similar to the clinically used verapamil. Another related constituent of "Tanshen" is neotanshinlactone (**76**), which has a lactone rather than *o*-quinone ring C (Figure 17).[44] This compound showed selective cytotoxicity against two estrogen receptor-positive (ER+) breast cancer cell lines, MCF-7 and ZR-75−1, and was 10-fold more potent than tamoxifen.[45] In initial SAR studies, 4-ethyl-neo-tanshinlactone (**77**) was even more potent (ED$_{50}$ = 0.45 and 0.18 μg/mL) than neotanshinlactone against these two cell lines and, in addition, potently inhibited (ED$_{50}$ = 0.1 μg/mL) the SK-BR-3 breast cancer cell, which is estrogen receptor negative (ER−), but overexpresses HER2 (HER2+).[46] In SAR analogue studies, the aromatic rings A and D were found to be important for antibreast cancer activity. In addition, certain ring C-opened analogues (**80**, **82**) retained activity and had increased selectivity toward specific breast cancer subtypes (Table 4).[47,48] The lead compound **76** was also tested in vivo against cancer

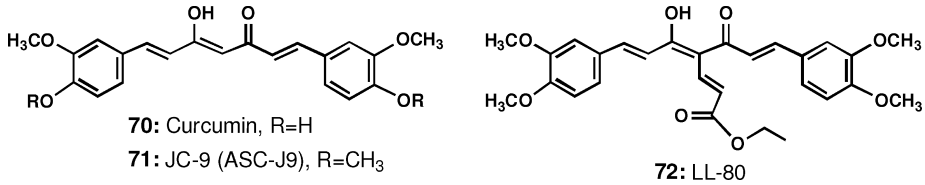


Figure 16. Structures of cytotoxic curcuminoids.

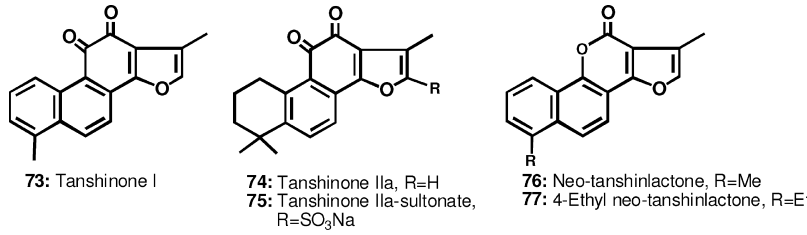


Figure 17. Structures of cytotoxic tanshinones and neo-tanshinlactones.

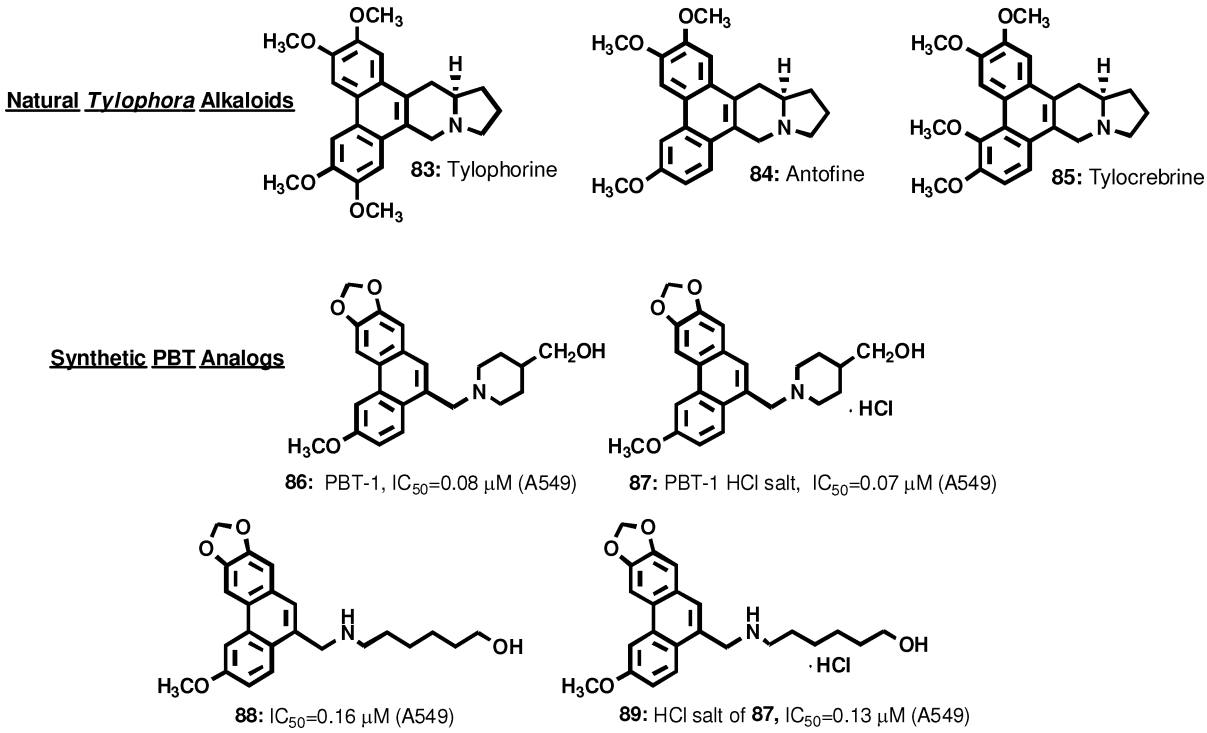
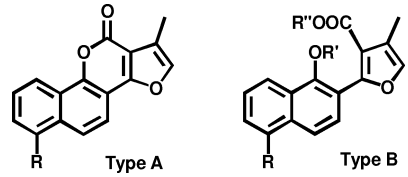


Figure 18. Structures of cytotoxic natural *Tylophora* alkaloids and synthetic PBT analogues.

Table 4. Cytotoxicity of Neo-tanshinlactone Analogues against Four Breast Cancer Cell Lines (in μg/mL)



cmpd	type	R	R′	R″	MCF-7 (ER+)	SK-BR-3 (HER2+)	ZR-75-1 (ER+,HER2+)	MDA MB-231 (ER−)
77	A	Et			0.2	0.1	0.1	>10
78	A	Pr			1.2	0.1	0.3	>10
79	A	OMe			2.3	0.2	0.1	6.4
80	B	Et	H	H	3.3	1.0	**0.3**	>10
81	B	Et	Me	Me	2.5	1.2	1.3	2.3
82	B	OMe	H	H	>20	3.5	**0.6**	>10

cell xenografts in mice. At 10 mg/kg, it remarkably delayed tumor growth compared to control and, thus, showed significant and selective antitumor activity against the human ZR-75-1 breast ductal carcinoma xenograft.[49]

Phenanthrene-Based Tylophorines: PBT-1 and Its Analogues. Tylophorine (**83**), antofine (**84**), and other phenanthroindolizidine alkaloids found in the genus *Tylophora* are collectively known as *Tylophora* alkaloids (Figure 18). Several such compounds have shown activity against various cancer cell lines, including refractory cancers. One of these compounds, tylocrebine (**85**), reached anticancer clinical trials, but failed due to central nervous system (CNS) toxicity.[50] Structurally simplified phenanthrene-based tylophorine (PBT) analogues (**86**−**89**, Figure 18), which lack the indolizidine ring system found in the natural product leads, were synthesized through an efficient five-step route.[51] The new compounds have a core phenanthrene substituted with an amino-substituted methylene at the 9-position. The amine group could be an alkylamino, pyrrole, piperidine, or piperazine with a terminal carboxy or preferably a hydroxy group.[52,53] *N*-(2,3-Methylenedioxy-6-methoxyphenanthr-9-ylmethyl)-L-piperidinemethanol (**86**, PBT-1) emerged as the lead compound from SAR studies. It showed good cytotoxic activity against various cancer cell types with IC_{50} values ranging from 0.04 to 0.07 μM. Its hydrochloride salt (**87**) also showed moderate in vivo activity against A549 lung cancer

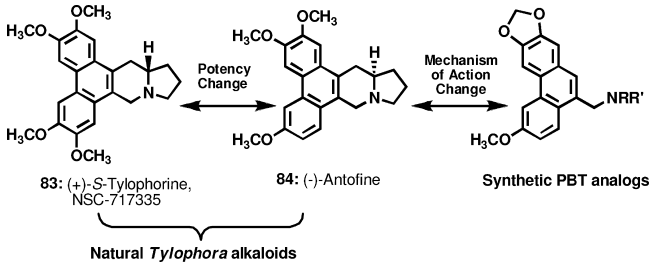
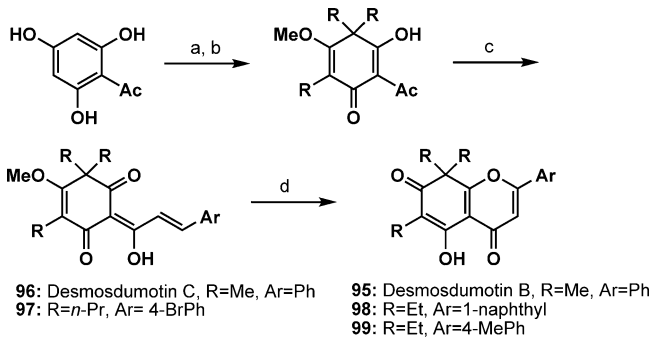


Figure 19. Mechanistic comparison of *Tylophora* alkaloids.

Scheme 1. Synthesis of Desmosdumotins B and C and Analogues[a]



[a] Reagents: (a) NaOMe, RI; (b) $TMSCHHN_2$; (c) ArCHO, KOH; (d) I_2, DMSO then BBr_3.

xenografts in mice, without CNS toxicity.[52] Interestingly, mechanistic studies showed that natural *Tylophora* alkaloids and the structurally related synthetic PBT analogues do not share the same mechanism of action (Figure 19).[53] Additional studies showed that **86** induced cell cycle arrest at the G2/M phase, accompanied by accumulation of cyclin B1 and activation of the MAPK signaling pathway, similar to paclitaxel.[54] In addition, **86** induced apoptosis by inactivating Akt and inhibiting the NF-κB pathway.[54] Lead PBT compound **86** or a new analogue will likely be a good candidate for anticancer clinical trials.

Conjugated Paclitaxel Analogues. Figure 20 shows examples of various conjugated paclitaxel analogues. First, a taxoid (either paclitaxel or cephalomannine) was conjugated at its 2′- and/or 7-position through an imine linkage to a 4β-amino-4′-*O*-demethylepipodophyllotoxin derivative.[55] Several compounds (e.g., **90**) showed cytotoxic activity comparable to or better than the unconjugated epipodophyllotoxin and showed enhanced activity against paclitaxel-resistant cancer cell lines.[55] Similarly, paclitaxel was also conjugated with a camptothecin derivative.[56] Compared with camptothecin, all of the new conjugates (e.g., **91**−**93**) showed higher cytotoxicity but lower inhibition of topo I. Against HCT-8 cancer cells, the conjugates were also more active than paclitaxel, suggesting a different spectrum of activity and, thus, possibly a novel mechanism of action.[56] Several different dietary antioxidants (including vitamin E, curcumin, dehydrozingerone, 4-methylumbelliferone, and others) were conjugated to the 2′-hydroxy group in the paclitaxel side chain through an ester linkage.[57] Many of the compounds showed selective cytotoxicity against certain cancer cell lines, particularly the 1A9 and KB cell lines. The paclitaxel−vitamin E conjugate (**94**) with a glycine ester salt at the C-7 OH of paclitaxel exhibited notable inhibition against pancreatic cancer (Panc-1) cells with a lesser effect on the normal (E6E7) cell line and is a good candidate for further studies.[57]

Chromenones: Desmosdumotin and Protoapigenone Analogues. Desmosdumotins B (**95**) and C (**96**) are bioactive flavanoids from *Desmos dumosus*. A short efficient route to both compound types was established (Scheme 1). Among **96**-type chalcones, a 4-bromo-3′,3′,5′-tripropyl analogue (**97**) was the most potent, with EC_{50} values of 0.9−2.3 μg/mL against seven different cancer cell lines, compared with 3.0−11.1 μg/mL for desmosdumotin C.[58] Among **95**-type flavones, the naphthyl-substituted **98** showed potent cytotoxicity against all six cancer cell lines tested, with EC_{50} values of 0.2−0.6 μg/mL. 6,8,8-Triethyl-substituted compounds (e.g., **99**), particularly when coupled with 4′-methyl or -ethyl substituents on the pendant phenyl ring, showed notable cytotoxicity (EC_{50} 0.03 and 0.025 μg/mL, respectively) and selectivity against vincristine-resistant KB cell lines (KB/KB-vin >460 and 320, respectively).[59] Protoapigenone (**100**) is structurally related to **95**, but has an unusual nonaromatic B-ring with a hydroxy group on the C-1′ position. The first total synthesis of this compound also allowed for preparation of modified analogues (Scheme 2). Initial SAR study showed that changing the C-5 and C-7 A-ring hydroxy groups to methoxy groups (e.g., **101**) or changing the phenyl A-ring to a naphthyl ring system (**102**) increased the cytotoxicity when compared to **100**. Analogues **101** and **102** had comparable potency to that of doxorubicin against liver and breast cancer cell lines, respectively.[60]

Kalanchosides. Three new bufadienolides (kalanchosides A−C, **103**−**105**) were isolated from the medicinal herb *Kalachoe gracilis*. These compounds showed remarkably potent cytotoxicity against a HTCL panel, particularly against the A549 lung cancer cell line, where the EC_{50} value of **103** was 0.5 ng/mL (Table 5).[61]

Summary of the Highlights of Antitumor Agents Discovered by NPRL in Clinical Trials and in Preclinical Studies. Numerous compounds with potent cytotoxicity have been discovered as new drug candidates through studies in the author's NPRL

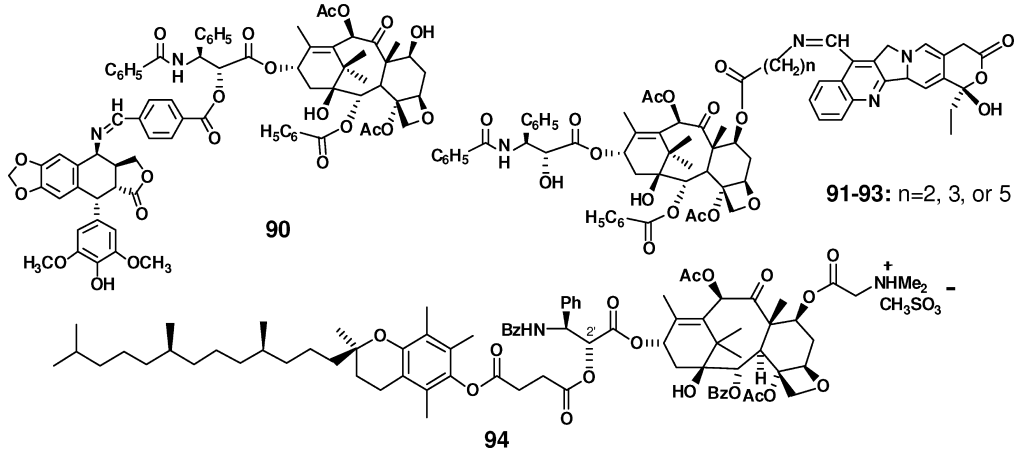
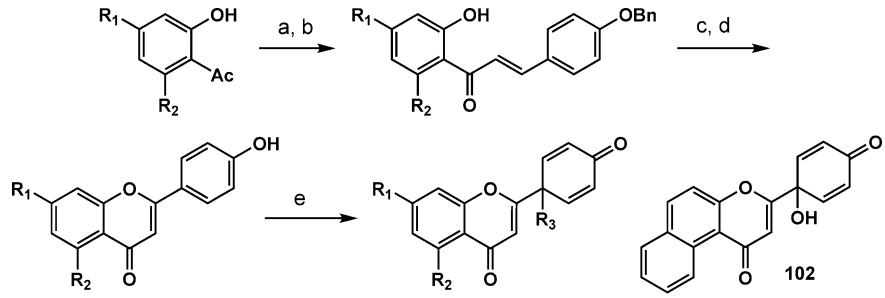


Figure 20. Structures of conjugated paclitaxel analogues.

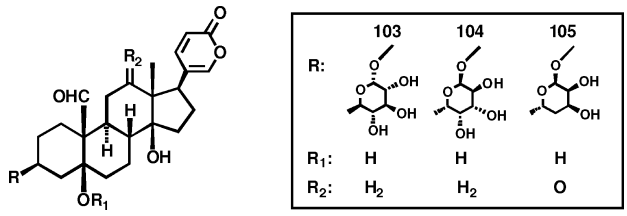
Scheme 2. Synthesis of Protoapigenone and Analogues[a]



100: Protoapigenone, R_1=R_2=R_3=OH
101: R_1=OMe, R_2=R_3=OH
102: see separate structure

[a] Reagents: (a) MOMCl, K_2CO_3; (b) KOH, 4-OBn-PhCHO; (c) I_2, DMSO or Py; (d) 10% Pd/CC, H_2; (e) TAIB, CH_3CN/H_2O for R_3 = OH or MeOH for R_3 = OMe; (f) 15% HCl/*i*-PrOH.

Table 5. Cytotoxicity of Kalanchosides



	IC_{50} (μg/mL) for 3 days continuous exposure						
cmpd	KB	KB-VIN	A549	1A9	PC-3	HCT-8	A431
103	0.003	0.003	0.0005	0.0008	0.002	0.006	0.007
104	0.005	0.013	0.001	0.007	0.010	0.015	0.022
105	0.016	0.026	0.006	0.012	0.025	0.045	0.055

and have been reviewed before in this journal.[62] A summary and highlights in regard to the above compounds are given in Figure 21.

Antimalarial Agents. *Artemisia annua* was long used as a medicinal plant for malaria fever, and artemisinin (**106**) ("Qing Hao Su"), isolated from this plant, was used as a safe and effective cure for malaria in mainland China.[63] Artemether (**107**) and arteether (**108**) are semisynthetic derivatives that are used clinically to treat drug-resistant malaria and as a second-line therapy for severe malaria cases, respectively.[64] A simpler endoperoxide (C−O−O−C-C═C) analogue (**109**) synthesized from α-santonin was not active, which showed that the unique 1′,2′,4′-trioxane ring (C−O−O−C−O−C) could be quite specific for activity, because a simpler cyclic epoxide ring was not adequate.[65] Subequent synthetic studies to explore this issue resulted in several desethanoqinghaosu analogues, both cyclic peroxide lactone (**110**) (C−O−O−C−O−C−O−C═O)[66] and nonlactone (**111**, **112**) (C−O−O−C−O−C−O) compounds,[66] and 12-deoxo (**113**, **114**) (C−O−O−C−O−C−O−C)[67] analogues. All of these compounds were less potent than **106**. However, tricyclic 1′,2′,4′-trioxane acid hydrolysis products (**115**, **116**) of **106**, in which the lactone ring was opened, were equipotent to the tricyclic parent compound. Thus, the ethane bridge forming the fourth ring is essential for potent activity and likely imposes a strict steric structure on the 1′,2′,4′-trioxane ring.[68] More recent studies by Posner et al. with a structurally related compound (**117**) have supported these earlier findings.[69] Compounds of interest are shown in Figure 22.

Antifungal Agents. Anthracenediones substituted with 2′,3′-epoxypropylamino groups were found to be potent antibacterial and antifungal agents. The compounds combined structural features of mitoxantrone (**118**), an anthracenedione antineoplastic agent, and teroxirone (**119**), an experimental triepoxide antitumor agent (Figure 23). 1,4-Di-(2,3-epoxypropylamino)anthracenedione (**120**) had a minimum inhibitory concentration (MIC) of less than 0.13 ppm against *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Aspergillus niger*, and *Aureobasidum pullulans*, but was less active against *Ps. aerugenosa* (MIC = 63 ppm) and *Escherichia coli* (MIC = 250 ppm).[70] Compound **120** was licensed by Rohm and Haas Company (Philadephia, PA) for use as an antifungal agent.

Anti-AIDS Agents. The life cycle of human immunodeficiency virus (HIV), the causative agents of AIDS, offers various points for chemotherapeutic attack. Many anti-HIV agents inhibit the actions of the viral enzymes, including reverse transcriptase (nucleoside and non-nucleoside reverse transcriptase inhibitors, NRTIs and NNRTIs), protease (protease inhibitors, PIs), and integrase (integrase inhibitors, IN). Drug/drug candidates that inhibit other viral processes include maturation inhibitors (MIs), which inhibit virus budding/maturation, and entry inhibitors, which inhibit viral adsorption, chemokine coreceptor binding, or virus−cell fusion. The NPRL's anti-AIDS research program focuses on discovering lead natural products with promising anti-HIV activity, which can offer new structural and mechanistic classes of drug/drug candidates, particularly as viral resistance to currently used agents is a growing problem and limits therapeutic options. The earliest work led to the discovery of four new tetragalloylquinic acids, which at least in part inhibited HIV RT transcriptase activity.[71] Continued studies have led to numerous natural products from various chemical classes with promising anti-HIV activity, based primarily on initial results from a screening to determine the level of HIV infection in treated cells. Examples (**121**−**131**) are given in Table 6.[72–92] More detailed discussion will be given on two compound classes: betulinic acid derivatives and suksdorfin derivatives.

Betulinic Acid Derivatives. 3-*O*-(3′,3′-Dimethylsuccinyl)-betulinic Acid (DSB, Bevirimat): Maturation Inhibitors. A prior review[93] described the identification of the triterpene betulinic acid (**130**) as an anti-HIV lead compound, followed by the identification of its ester analogue, 3-*O*-(3′,3′-dimethylsuccinyl)betulinic acid (DSB, **133**) (Figure 24), as the first HIV maturation inhibitor, through SAR modification, mechanism of action, and preclinical studies. A brief description of these studies will be given here.In 1994, **130** was isolated as anti-HIV principle from leaves of *Syzigium claviflorum* ("Pang Hua Chih Nan").[91] It is also found in the bark of the London plane tree (*Platanus acerifolia*), and its precursor betulin (**134**) in the bark of white birch (*Betula alba*) (Figure 24). Among a series of 3-acyl derivatives of **130**, **133** was found to be the most potent compound (Table 7, **135**−**140**).[94] It was licensed to Panacos Inc. (Watertown, MA), renamed PA-457 and then Bevirimat, and subjected to intensive preclinical studies. It was a potent inhibitor of primary HIV-1 isolates in vitro, retained activity against virus isolates resistant to NRTIs, NNRTIs, and PIs, and also showed synergistic effects with other AIDS drugs. In

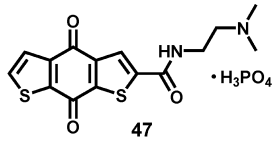
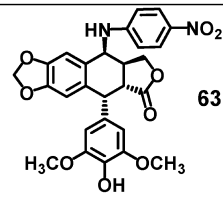
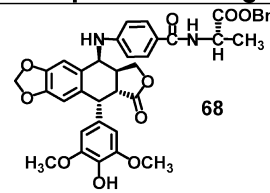
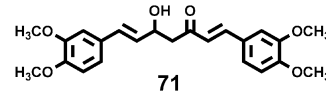
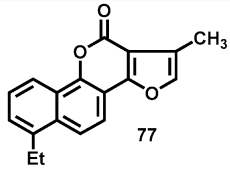
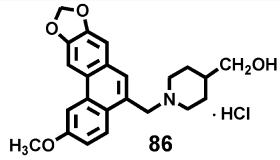
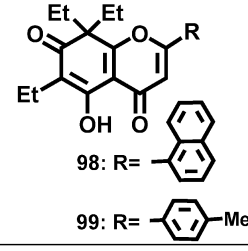
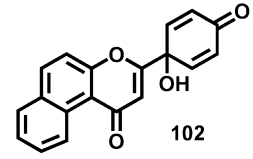
Dithiophene Analog	**GL-331**	**4β-Amino Etoposide Analog**	**JC-9 (ASC-9)**
 47	 63	 68	 71
• **In Vitro Activity:** Induced nearly complete differentiation at concentration of 0.02 μM • **Mechanism:** Showed great enhancement of all-trans-retinoic acid (ATRA)-induced differentiation in H9 cells • **Drug Development:** Clinical trials are being planned	• **Mechanism:** DNA topo II inhibitor with superior activity profile including overcoming multidrug resistance to etoposide both in vitro & in vivo • **Drug Development:** Succeeded in Phase I clinical trials by M.D. Anderson Cancer Center Marked antitumor efficacy in 4 tumor types (non-small & small cell lung, colon, head/neck) Patented and licensed to Genelabs Technologies, Inc. CA in 1992 Phase II clinical trials in US are being planned	• **In Vitro Activity:** Showed better activity profiles than GL-331; Showed superior inhibition against both KB and KB-7d cells • **Mechanism:** DNA Topo II inhibitor • **Drug Development:** In vivo & preclinical studies are in progress	• **In Vivo Activity:** In vivo active against liver & bladder cancers • **Mechanism:** Enhances androgen receptor degradation • **Drug Development:** Patented and licensed to Androscience Corporation of San Diego, CA Succeeded in Phase II clinical trials for treating acne in 2008 Clinical trials for treating prostate cancer are being planned
4-Ethyl-neo-tanshinlactone	**PBT-1**	**Desmosdumotin B Analogs**	**Protoapigenone Analog**
 77	 86	 98: R= 99: R= Me	 102
• **In Vitro Activity:** Selective against two ER+ breast cancer cell lines MCF-7 and ZR-75-1 Potent activity against ER-, HER2+ breast cancer cell line SK-BR-3 **In Vivo Activity:** Active against human ZR-75-1 breast ductal carcinoma xenograft	• **In Vitro Activity:** IC_{50} = 0.07 μM (A549) • **In Vivo Activity:** Significant tumor inhibition on day 5 & moderate growth inhibition from day 9 to day 29 w/o overt toxicity [in vivo activity against A549	• **In Vitro Activity:** Selective cytotoxicity against KB-Vin **Cmpd 98**: IC_{50} = 0.03 μg/mL **Cmpd 99**: Cytotoxic against MCF-7 • **Drug Development:** Potential anticancer agents	• **In Vitro Activity:** Cytotoxic against Hep3B, MDA-MB-231, and MCF-7 with IC_{50} 0.30, 0.39, and 0.66 μg/mL, respectively Comparable potency to doxorubicin against liver and breast cancer cell lines • **Drug Development:** Clinical trials are being planned

Figure 21. Summary of the highlights of antitumor agents discovered by NPRL in clinical trials and preclinical studies.

mechanistic studies, **133**-treated HIV virions showed immature, spherical cores.[95] Studies suggested that **133** interferes with normal gag processing, which is necessary to form mature infectious virus particles. Indeed, further research proved that **132** disrupts cleavage at the CA-SP1 junction of the gag precursor protein and disrupts normal capsid condensation. The resulting virus particles are defective and noninfectious. In summary, **132** was found to be the first-in-class HIV maturation inhibitor, targeting the CA-SP1 region of gag.[88,95] Compound **133** was easily formulated in salt form with good oral availability, was eliminated via glucuronidation, and was active in a SCID mouse model.[96] In phase I clinical trials, it was safe and well tolerated with a good half-life and plasma levels.[97] In phase II clinical trials, **133** reduced viral load significantly (mean of -1.18 $\log_{10}$ copies/mL after 14 days of treatment) with good plasma levels found with a tablet formulation.[97] It has now been licensed to Myriad Pharmaceuticals (Salt Lake City, UT), which has renamed the compound MPC-4326 and is planning phase III clinical trials for 2009/2010. The U.S. FDA granted "Fast-Track" new drug development status for **133**, for treatment of HIV in combination with already approved drugs or possibly as a first-

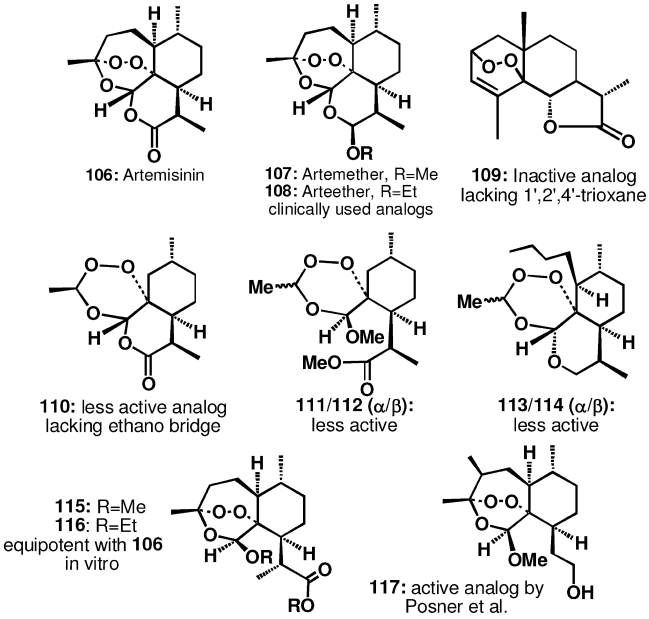


Figure 22. Structures of artemisinin and inactive, less active, and equipotent analogues.

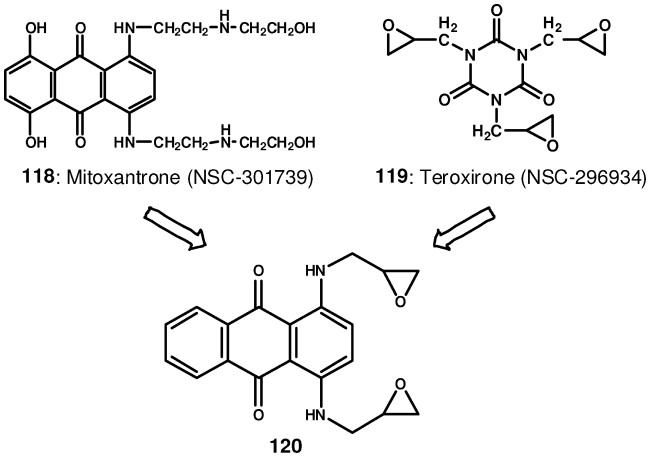


Figure 23. Structure of antibacterial/antifungal agent licensed by Rohm & Haas.

line therapy. Figure 25 summarizes the status of the clinical development of **133**.

(3′-Monomethylsuccinyl)betulinic Acid (MSB) Analogues. More recently, the 3′ S-methyl group was found to be the main contributor to the extremely high anti-HIV potency, because 3′*S*-MSB (***S*-141**) was much more active than 3′*R*-MSB (***R*-141**) (Table 8).[98]

C-28 Amide-Substituted Analogues: Entry Inhibitors. For anti-HIV maturation activity, the C-3 position of betulinic acid analogues was found to be the pharmacophore for anti-HIV maturation activity (Figure 26). For optimal potency, the analogue should have a C-3 acyl side chain with the proper length, terminal carboxylic acid moiety, and dimethyl substitution at the C-3′ position for optimal potency.[93,97] However, prior studies by Soler et al. showed that betulinic acid derivatives substituted with ω-aminoalkanoic acid at the C-28 position, e.g., RPR103611 (**142**, Figure 27), showed anti-HIV activity in the nanomolar range, by interfering with the virus−cell fusion process.[99] Thus, the C-28 position of betulinic acid is the pharmacophore for anti-HIV entry activity (Figure 26).[99,100] Addition of two ω-aminoalkanoic acids ($m = 7-10$, $n = 3$ or 4) resulted in optimal activity. Both statine and L-leucine, as the terminal amino acid, gave potent analogues, as evaluated in the MAGI assay for HIV-1 entry inhibitors.[101] The exact target of these triterpene entry inhibitors has not been determined, although viral mutations that confer resistance were found in gp41 for RPR103611 and gp120 for its stereoisomer IC9564 (**143**, Figure 27).[100]

C-3, C-28 Disubstituted Inhibitors: Bifunctional Inhibitors. Addition of an appropriate acyl side chain at C-3 and amide side chain at C-28 to betulinic acid results in derivatives that inhibit both HIV entry and maturation.[100] In SAR studies, the lead compound **A12-2** (**144**; dimethylsuccinyl at C-3, 7-aminoheptanoic acid at C-28) was at least 20 times more potent at inhibiting HIV replication than either **130** (C-3, but not C-28 substituted; maturation inhibitor only) or **143** (C-28, but not C-3 substituted; entry inhibitor only), as well as demonstrating both antimaturation and antientry activities (Figure 28).[102] In SAR studies, smaller and bulkier C-3 acyl side chains were detrimental to activity.[103] At the C-28 position, forming the amide bond by using a cyclic secondary amine, such as piperidine, increased metabolic stability.[104] Regarding the triterpene molecular scaffold, moronic, ursolic, and oleanolic acid analogues were active, while glycyrrhetinic and lithocholic acid analogues were not.[103,105] In fact, moronic analogue **145**, which is substituted with a 3′,3′-dimethylsuccinyl ester at C-3 and L-leucine amide at C-28, showed better potency than bevirimat against several HIV strains (EC_{50} values against NL4-3 were 0.0085 μM/0.096 μM and against PI-R were 0.021 μM/0.43 μM). Thus, **145** could be another promising clinical trial candidate in the triterpene class.

Suksdorfin Derivatives. Dicamphanoylkhellactone (DCK) Analogues. Bioactivity-directed fractionation using a p24 antigen ELISA assay for HIV_{IIIB} replication in H9 lymphocytes led to the isolation of the natural coumarin suksdorfin (**132**) as an anti-HIV principle ($EC_{50} = 1.3$ μM, TI >40) from *Lomatium suksdorfii*.[106] Replacing the two natural acyl groups with various esters led to the synthesis of the new anti-HIV lead 3′,4′-di-*O*-(−)-camphanoyl-(+)-*cis*-khellactone (**146**, DCK) (Figure 29), which had greatly increased potency ($EC_{50} = 0.049$ μM, TI >328).[77,106] Compound **146** and its derivatives could be prepared efficiently through a synthetic route developed using a Sharpless asymmetric dihydroxylation (Scheme 3).[107,108] In initial SAR studies, methylation at the 4- or 5-position of the coumarin ring greatly increased potency, resulting in EC_{50} and TI values of 0.006 μM/6600 (4-MeDCK, **148**) and 0.0086 μM/>2000 (5-MeDCK, **149**) (Table 9).[109] 6-MeDCK (**150**) was much less active, and 3-MeDCK (**147**) was inactive. Adding a methoxy group at position 3, 4, or 5 (**151**−**153**, respectively) resulted in retained activity relative to **146**, while 6-OMeDCK (**154**) was inactive (Table 9). Disubstitution or monosubstitution with larger alkyl or phenyl groups was less favorable or unfavorable.[109] In an effort to enhance water solubility

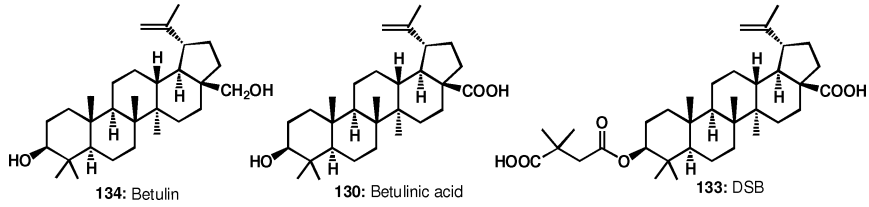
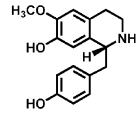
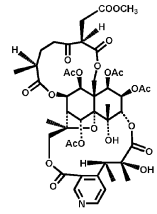
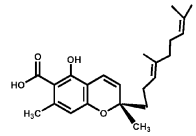
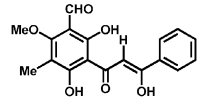
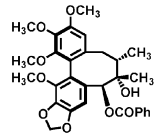
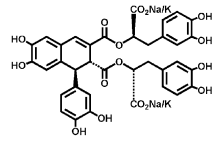
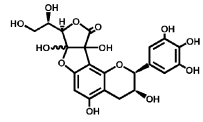
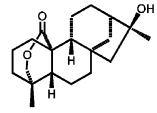
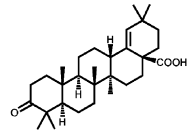
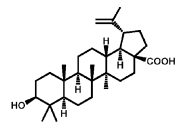
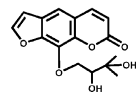
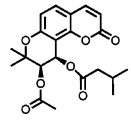
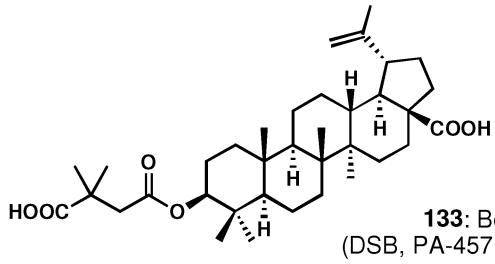


Figure 24. Structures of betulinic acid, its natural precursor betulin, and its synthetic analogue DSB.

Table 6. Examples of Anti-HIV Natural Products Identified by the NPRL[72–92]

cmpd	name	structure	plant source	chemical class	anti-HIV EC_{50} (μM)	TI ($IC_{50} \div EC_{50}$)
121	(+)-1*R*-cocluaurine[72]		*Nelumbo nucifera*	alkaloid	2.8	125
122	triptonine B[73]		*Tripterygium hypoglaucum*	sesquiterpene alkaloid	<0.1 (μg/mL)	>1,000
123	daurichromenic acid[78]		*Rhododendron dauricum*	chromane	0.015	3,710
124	2-methoxy-3-methyl-4,6-dihydroxy-5-(3'-hydroxy)cinnamoyl-benzaldehyde[79]		*Desmos spp*	flavonoid	0.067	489
125	gomisin G[83]		*Kadsura interior*	lignan	0.011	600
126	sodium and potassium salts of caffeic acid tetramers[85]		*Arnebia euchroma*	caffeic acid tetramer	1.9	33.3
127	8-*C*-ascorbyl-(-)-epigallocatechin[86]			tea polyphenol	4	9.5
128	neotripterifordin[87]		*Tripterygium wilfordii*	diterpene	0.025	125
129	moronic acid[88]		Brazilian *Propolis*	triterpene	<0.22	>186
130	betulinic acid[91]		*Syzygium claviflorum*	triterpene	1.4	9.3
131	heraclenol[75]		*Ferula sumbul*	coumarin	0.38	870
132	suksdorfin[77]		*Lomatium suksdorfii*	coumarin	1.3	40



133: Bevirimat
(DSB, PA-457, or MPC-4326)

- **133** is a First-in-Class HIV maturation inhibitor, targeting the CA-SP1 region of Gag.
- Gag processing is necessary for formation of mature, infectious viral particles. **133** disrupts the cleavage at CA-SP1 junction, resulting in the production of non-infectious HIV virus.
- **133** succeeded in Phase IIa (8/2005) and Phase IIb (12/2008) clinical trials as an anti-AIDS drug.
- Phase III clinical trial in 2010 (renamed as **MPC-4326**) is under planning by Myriad Pharmaceuticals Inc.

Figure 25. Summary of clinical development of **133**, an anti-AIDS compound discovered by NPRL.

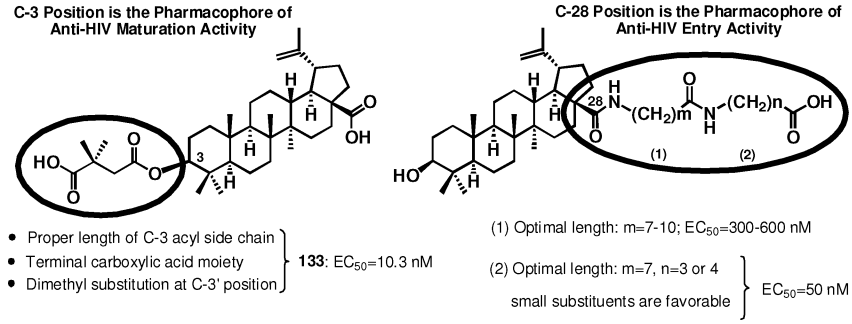


Figure 26. Pharmacophores for anti-HIV maturation versus entry inhibitory **130** analogues.

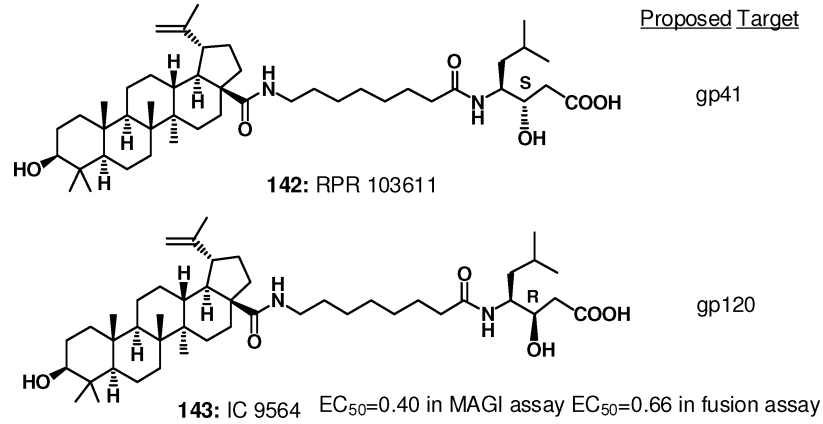
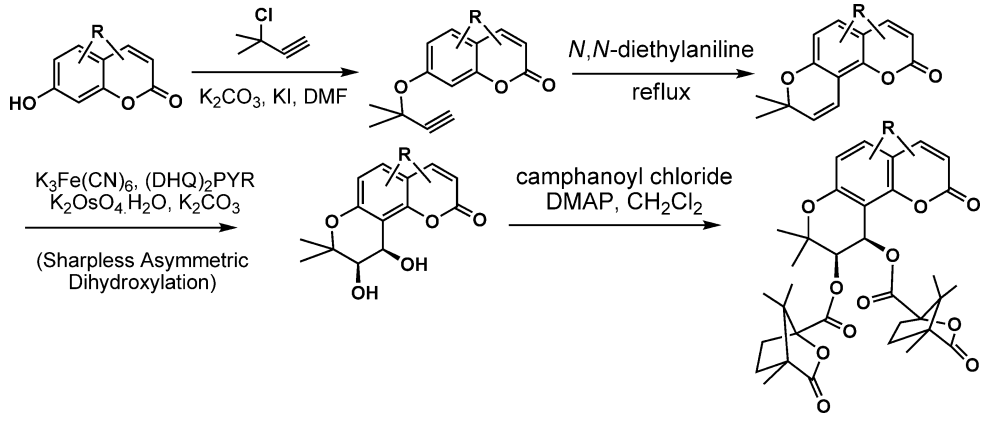


Figure 27. 130-Derived HIV-1 entry inhibitors.

Scheme 3. Synthesis of DCK Analogues



and oral bioavailability of DCK analogues, methyl groups substituted with various polar groups ($-CH_2X$) were added.[110] While addition of aminomethyl or diethylaminomethyl groups at the C-3 position of 4-methylDCK decreased potency, both bromomethyl

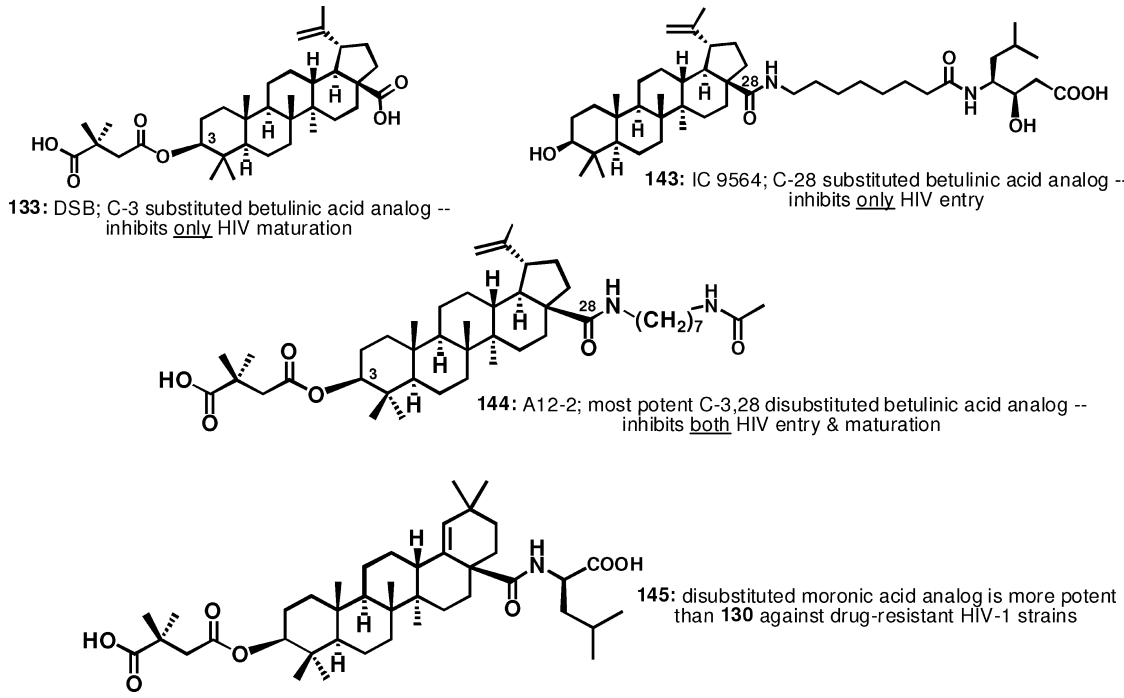
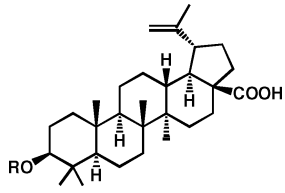
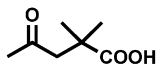
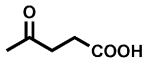
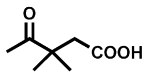
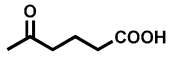
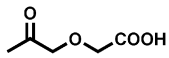
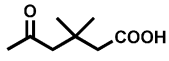
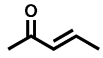


Figure 28. Comparison of C-3 mono-, C-28 mono-, and C-3,28 disubstituted triterpene HIV-1 inhibitors.

Table 7. Anti-HIV Activity of **130** Analogues in H9 Cells



cmpd	R	IC_{50} (μM)	EC_{50} (μM)	TI
130	H	13.0	1,4	9.3
133 (DSB)		7	0.00035	20,000
135		16	4.0	4
136		15.9	2.7	6.7
137		12.8	0.044	292
138		11.7	0.01	1,170
139		4.5	0.003	2,000
140		48	19	2.5

and hydroxymethyl groups were favorable. Indeed, 3-bromomethyl-4-methylDCK (**155**) showed impressive EC_{50} and TI values of 0.000 11 μM/186 000 (Figure 30).[110] However, 3-hydroxymethyl-4-methylDCK (**156**, HMDCK) (EC_{50} 0.0042 μM, TI 6000) was selected as a clinical trial candidate and licensed by Panacos Pharmaceuticals, based on better oral bioavailability in rats. In mechanistic studies, **146** and **156** were found to inhibit DNA-dependent DNA polymerase activity of the viral reverse transcriptase (RT). However, unlike traditional RT inhibitors that block generation of single-stranded DNA from the RNA template, **146**

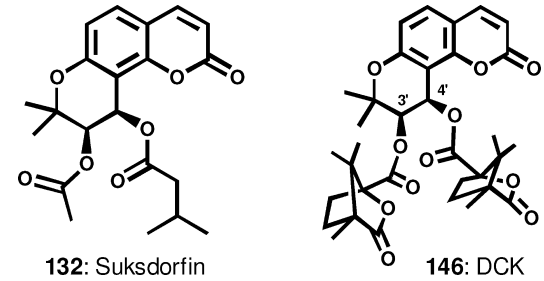
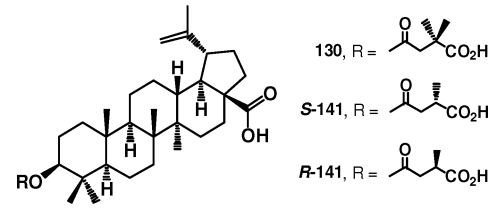


Figure 29. Structures of anti-HIV natural coumarin suksdorfin and synthetic analogue DCK.

Table 8. Anti-HIV Activity of Monomethylsuccinyl Analogues of **130**



cmpd	IC_{50} (μM)	EC_{50} (μM)	TI
***S*-141**	33	0.0087	6274
***R*-141**	>40	0.12	>961
***S*-141 + *R*-141**	>40	0.016	>5323
133	>40	0.0013	>30 555
AZT	1870	0.034	55 330

and its analogues inhibit the production of double-stranded vial DNA from the single-stranded DNA intermediates. The exact binding site of these compounds has not yet been determined, but is possibly in the p51 subunit of HIV RT, where **146** binding could interfere with second strand transfer.[111] Regardless, the unique mechanism of action makes **146** or future analogues potentially clinically useful.In SAR studies, replacing an oxygen atom in the lactone ring of **146** with sulfur or nitrogen led to analogues with significant potency, with EC_{50} values in the lower micromolar to

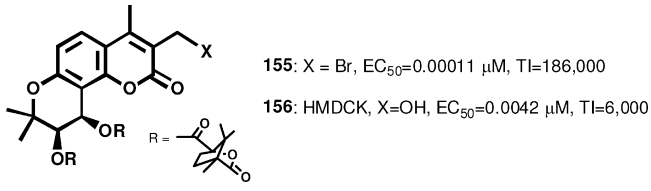


Figure 30. Potent disubstituted analogues of **146**.

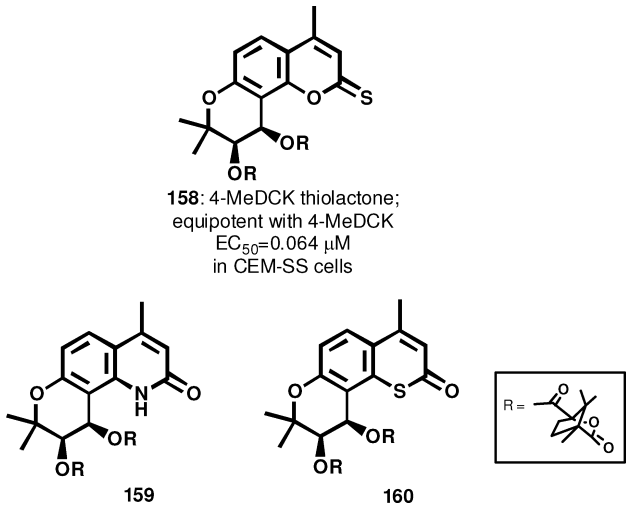
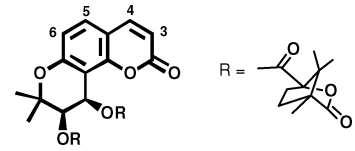


Figure 31. Biostereoisomeric analogues of **146**.

nanomolar range.[112–114] For example, replacing the carbonyl oxygen of 4-methyl-DCK with sulfur (−OC═S) giving **158** (4-MeDCK thiolactone) led to no loss or improvement in potency when assayed in CEM-SS cells (Figure 31).[112] Similarly, replacing the alcoholic oxygen of the lactone with nitrogen (−NHC═O, **159**)[113] or sulfur (−SC═O, **160**)[114] led to equipotent or slightly more potent compounds, compared with **146** (−OC═O).

Dicamphanoylpyranochromone (DCP) Analogues. Another significant structural variation was to synthesize 3′*R*,4′*R*-di-*O*-(−)-camphanoyl-2′,2′-dimethyldihydropyrano[2,3-*f*]chromone (DCP) analogues, which are positional isomers of DCK.[115] Among a series of mono- and disubstituted DCP derivatives, several compounds (**161**−**166**) exhibited extremely high anti-HIV activity in the non-drug-resistant strain assay, with EC_{50} values ranging from 0.00032 to 0.0057 μM and remarkable therapeutic indexes (TI) ranging from 5.6×10^3 to 1.16×10^5 (Table 10), which were similar to those of **148** (EC_{50} 0.0059 μM, TI 6.6×10^3) and better than those of **146** (EC_{50} 0.049 μM, TI 328). Even more promisingly, some DCP analogues also showed activity against a multi-RT inhibitor resistant strain, HIV-1 RTMDR1, whereas most DCK analogues did not. An ethyl group was the optimal C-2 substituent for activity against non-drug-resistant and multidrug-resistant HIV strains. Thus, the most significant compound was 2-Et-DCP (**162**), which showed the best anti-HIV activity in both assays, including an EC_{50} value of 0.06 μM and TI of 718 against the multi-RT inhibitor resistant HIV-1 strain (resistant to AZT, ddI, nevirapine, and other NNRTIs). In addition, 2-substituted DCPs were less toxic to cells than the unsubstituted or 3-substituted compounds. Due to their activity against drug-resistant HIV strains, DCP analogues may well be more promising than DCK analogues for further development as clinical trial candidates. Figure 32 summarizes the status of the preclinical development of DCK and DCP derivatives.

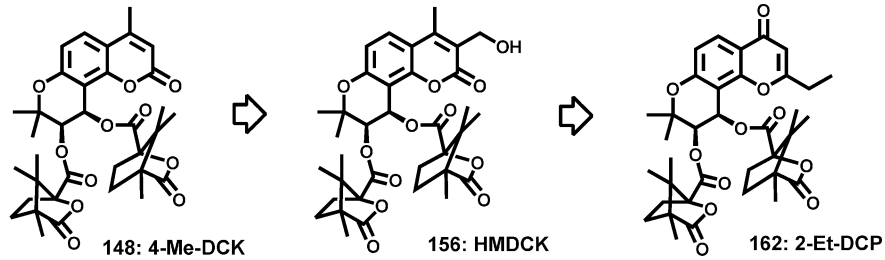
Table 9. Effect of Monomethylation or -methoxylation on Activity of DCK



cmpd	IC_{50} (μM)	EC_{50} (μM)[a]	TI
DCK (**146**)	>16.1	0.05	>328
3-Me (**147**)		no suppression	
4-Me (**148**)	>39	0.006	6600
5-Me (**149**)	>16	0.008	>2000
6-Me (**150**)	>16	0.21	>72
3-OMe (**151**)	>15	0.03	>533
4-OMe (**152**)	>15	0.05	>300
5-OMe (**153**)	>15	0.044	>350
6-OMe (**154**)		no suppression	
AZT		0.044	

[a] HIV replication in H9 lymphocytes.

Summary. Plant products still serve as an excellent source for modern drug discovery and development. Through a medicinal chemistry approach, natural products with low bioactivity or known compounds can be modified synthetically to improve their pharmacological profiles. Synthesis of new compounds must be accompanied by appropriate biological assay screening to successfully



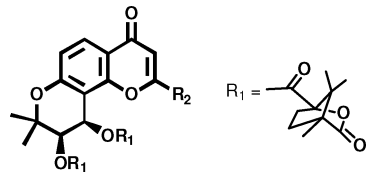
- Suksdorfin derivative with two camphanoyl moieties introduced at 3',4' positions
- EC_{50} and TI values are 0.006 μM and >6600, respectively
- Functions as Strand Transfer Inhibitor (STI), novel in NNRTI family

- EC_{50} and TI values are 0.004 μM & 6000, respectively
- Increased water solubility and oral bioavailability (F=15%)
- Potential to form prodrugs
- Lost anti-HIV activity towards RT-resistant virus

- DCP is a ring position isomer of DCK
- Retains activity in the multi-RT resistant strain (EC_{50} in HIV-1 RTMDR1: 0.06 μM)
- Further modification is in progress

Figure 32. Summary of preclinical development of DCK and DCP derivatives, anti-HIV compounds discovered by NPRL.

Table 10. Anti-HIV Activity of 2-Substituted DCPs



		HIV-1 IIIB			HIV-1 RTMDR1		
cmpd	R_2	IC_{50} μM	EC_{50} μM[a]	TI	IC_{50} μM	EC_{50} μM[a]	TI
161	Me	27.3	0.0031	8600	11.8	0.19	63
162	Et	37.2	0.000 32	116 200	43.1	0.06	718
163	*n*-Pr	>37.7	0.02	1860	37.7	0.14	272
164	*i*-Pr	33.4	0.07	483	>15	0.14	>111
165	CH_2OEt	15.1	0.1	151	12.5	0.37	34
166	C_6H_5	36	0.13	277	12.2	0.17	71
146 (DCK)		>16	0.049	>328	>16.1	12.1	1.3
148 (4-MeDCK)		>39	0.0059	>6600	>16	9.43	1.7

[a] HIV replication in H9 cells.

optimize a lead compound into a clinical trial candidate. As shown by the work described above, academic laboratories can indeed successfully accomplish the goals of bringing compounds into clinical trials.

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