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Lignans in treatment of cancer and other diseases[†]

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Abstract

Lignans are widely distributed in nature and display an impressive range of biological activities. The extensive pharmaceutical use of lignans is linked to their antitumor, antiviral, hepatoprotective, and platelet activating factor (PAF) antagonistic activities, among many others. This review article highlights selected pharmacologically active lignans, outlines their therapeutic relevance to the treatment of cancer and other diseases, and accentuates research on plant-derived lignans, particularly preclinical lead identification and optimization studies performed in the authors' Natural Products Laboratory (NPL). Antitumor and anti-HIV lignans have been discovered and developed in the NPL by using bioactivity-directed fractionation and isolation as well as rational drug design-based structural modification and analog synthesis approaches. Notably, a significant and ongoing project on podophyllotoxin and its semisynthetic analog etoposide has led to the development of a potent derivative designated GL-331. GL-331 has received investigational new drug (IND) approval from the FDA and is currently in clinical trials against various cancers, especially drug-resistant cancers. Further design, synthesis, and evaluation of GL-331-related analogs are discussed.

Introduction

Lignans are a well-known class of widespread natural products with huge structural and biological diversity. Lignans are widely distributed in numerous plant species and are present at different levels of abundance in all plant parts, including roots, rhizomes, hardwood, bark, stems, leaves, flowers, fruits, and seeds (Ayres and Loike, 1990). Lignans have also been found in the urine and blood of human and other mammals (Wahala et al., 1986). By definition, lignans are usually chemical assemblies of two phenylpropanoid (C_6-C_3) units, although higher oligomers have also been reported. Phenylpropanoid dimerization, oxygen incorporation, and skeletal functionalization may occur in different ways to give a great variety of structures. The principal lignan skeletons are listed in Figure 1. Due to their diverse biological activities, including antitumor, antiviral, hepatoprotective, platelet activating factor (PAF) antagonism, and other properties, lignans are of considerable pharmacological and clinical interest in the treatment of cancer and other diseases (Ríos et al., 2002).

The therapeutic use of lignans stems back to ancient times, when plant extracts containing lignans were used as folk remedies in various cultures (Kelly, 1954; Hartwell, 1954). The alcoholic extract of roots and rhizomes of *Podophyllum* species (Berberidaceae) was used to treat the bites of venomous snakes and as purgative, antihelminthic, vesicant and suicide agents in America approximately 500 years ago. The dried roots and stems of *Kadsura coccinea* (Schisandraceae), which contain at least ten dibenzocyclooctene-derived lignans (Li et al., 1985), are included in the Chinese Pharmacopoeia for treatment of rheumatoid arthritis and gastric and duodenal ulcers (Pharmacopoeia of the People's Republic of China, 1977). *Schisandra* (Schisandraceae)

[†]Antitumor Agents 229. For part 228, see Chang et al., J. Nat. Prod. 2003, 66, 1416–1420.

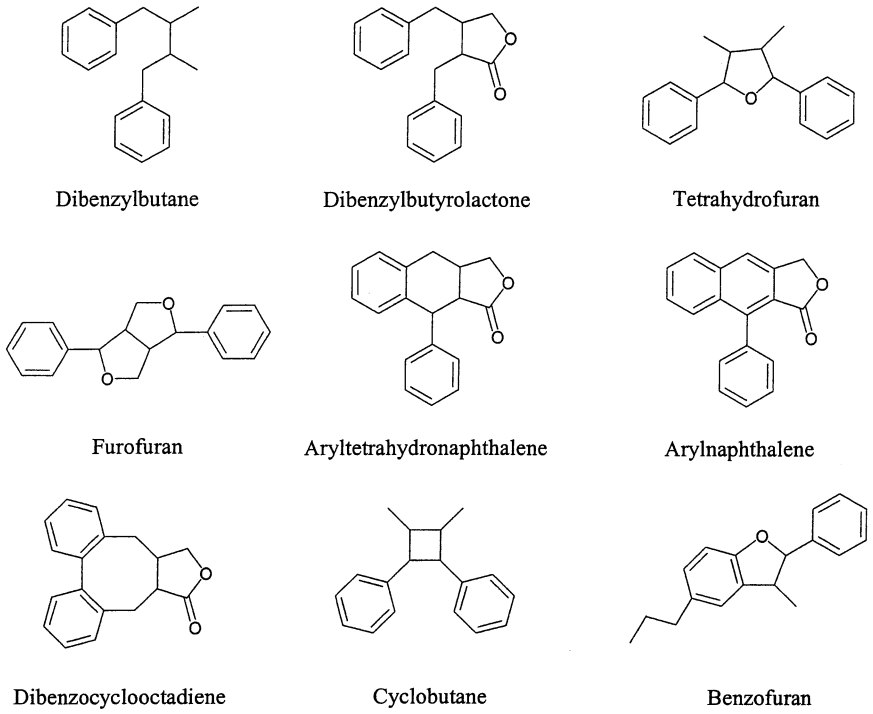


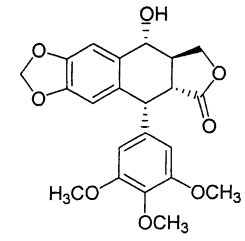
Figure 1. Representative skeletons of lignans.

fruits, which include lignans effective against chronic viral hepatitis and with protective action against liver damage by hepatotoxins, are used successfully to treat hepatitis (Ríos et al., 2002). The dried bark of *Fraxinus mandshurica* (Oleaceae), which contains at least eight different lignans (Tsukamoto et al., 1984a), and *Fraxinus japonica* was used as a diuretic, antipyretic, analgesic and antirheumatic in China and Japan, respectively. The bark of *Olea europaea*, from which a group of lignans has been isolated (Tsukamoto et al., 1984b), was used in the Eastern world as an antipyretic, antirheumatic, and tonifc agent, and the lignans are believed to be responsible for such properties.

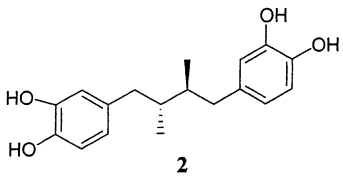
The isolation and identification of lignans from these traditional medicines have led to the discovery of several clinical drugs. Among them, podophyllotoxin (**1**), a cyclolignan abundant in *Podophyllum* species, is used as an antiviral agent for the treatment of *Condiloma acuminata* and other types of warts (von Krogh, 1981), and masoprocol (**2**), isolated from *Larrea tridentata*, is a useful topical agent for the prevention and treatment of pre-malignant skin lesion induced by actinic processes (Olsen et al., 1991).

Bioactive natural lignans can also serve as lead compounds for the development of new drugs through rational structural modification and analog synthesis, as is the case for podophyllotoxin. Three derivatives, etoposide (**3**), teniposide (**4**), and etopophos (**5**), which have improved pharmacological profiles over the parent compound podophyllotoxin, have been developed and are being used clinically in treatment of different types of cancer (Gordaliza et al., 2000a).

It appears that lignans represent a bioactive natural product class with diverse pharmacological actions in man and a promising prototype for the development of therapeutic agents. Previous reviews dealing with the biological activities of lignans (MacRae and Towers, 1984; Ayres and Loike 1990; Ríos et al., 2002) and periodic reviews covering most aspects of lignans (Whiting, 1985, 1987, 1990, Ward, 1993, 1995, 1997, 1999) have provided a comprehensive view of this compound class. In this review article, we aim to highlight representative lignans with pharmacological and clinical relevance to the treatment of cancer and other diseases and accentuate research performed in the authors' Natural Products Laboratory (NPL), particularly the synthesis and biological evaluation of etoposide analogs.



1



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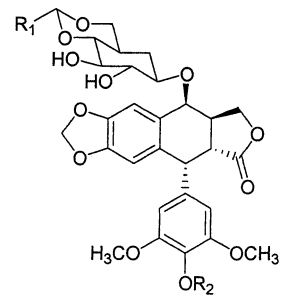


	R_1	R_2
3	CH_3	H
4		H
5	CH_3	

Antitumor lignans

The best-known antitumor lignan is podophyllotoxin (**1**), a bioactive component of *Podophyllum peltatum*, *P. emodi*, and *P. pleianthum*. Although often ignored due to the predominant attention devoted to the podophyllotoxin derivatives, it is worthwhile noting that many other antitumor lignans exist. Up to 1984, 33 lignans had been demonstrated to possess cytostatic or antitumor activity through either *in vitro* or *in vivo* methods (MacRae and Towers, 1984). In the following years, natural and synthetic lignans with antitumor activity have continued to be reported and their number has risen dramatically. Several representative skeletal types of antitumor lignans, particularly podophyllotoxin analogs, are discussed below.

Podophyllotoxin analogs

Although the therapeutic application of podophyllotoxin (**1**) itself as an anticancer drug has failed due to its severe toxicity, its semisynthetic glucosidic acetals, etoposide (**3**) and teniposide (**4**), are currently used clinically for treating various cancers. The impressive antitumor potency and clinical efficacy of **3** and **4** have prompted intensive research interest in new podophyllotoxin analogs. Numerous reviews related to the podophyllotoxin analogs have appeared in the literature. Some recent contributions include Zhang and Lee, 1994; Bohlin and Rosén, 1996; Imbert, 1998; Calixto et al. 1998; Damayanthi and Lown, 1998; Canel et al., 2000; and Gordaliza et al., 2000a.

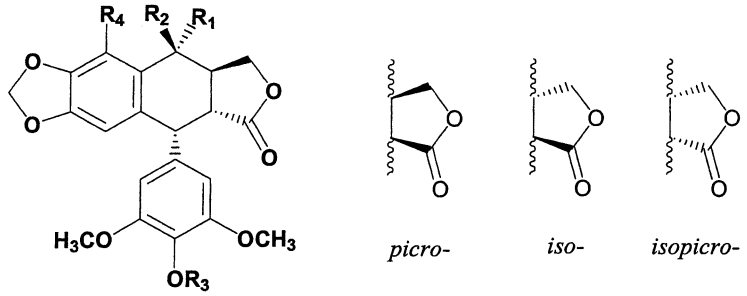
Structures

The podophyllotoxin analogs include several closely related structural classes, such as podophyllotoxin, deoxypodophyllotoxin, 4′-demethylpodophyllotoxin, 4′-demethylepipodo-phyllotoxin, α- and β-peltatins, and their D ring stereochemical variants, picro (2R, 3S), iso (2S, 3S), and isopicro (2S, 3R) derivatives (Figure 2). To date, most of the clinically relevant antitumor podophyllotoxin analogs are 4′-demethylepipodophyllotoxins (DMEP, e.g. etoposide and teniposide).

Mechanism of action

Primary molecular mechanisms underlying the antitumor activities of podophyllotoxin analogs include preventing the assembly of tubulin into microtubules or inhibiting the catalytic activity of DNA topoisomerase II (topo II), though other mechanisms and, in some cases, mechanisms that are still ambiguous are also involved.

Podophyllotoxin was found to inhibit assembly of the mitotic spindle and induce arrest of the cell cycle at mitosis by binding to tubulin, the fundamental monomeric sub-unit of microtubules. It shares the same binding site on tubulin with colchicine, the classic antimicrotubule agent. However, the binding of podophyllotoxin was more rapid and, in contrast to that of colchicine, reversible (Cortese et al., 1977). Many *Podophyllum*-derived compounds and other closely related compounds, such as peltatins, share this mechanism of action. In contrast, unlike podophyllotoxin, etoposide shows few effects on tubulin polymerization. Instead, it induces topo II-



	R_1	R_2	R_3	R_4
Podophyllotoxin	OH	H	CH_3	H
Deoxypodophyllotoxin	H	H	CH_3	H
4′-Demethylpodophyllotoxin	OH	H	H	H
4′-Demethylepipodophyllotoxin	H	OH	H	H
α-Peltatin	H	H	H	OH
β-Peltatin	H	H	CH_3	OH

Figure 2. Chemical structures of main podophyllotoxin analogs.

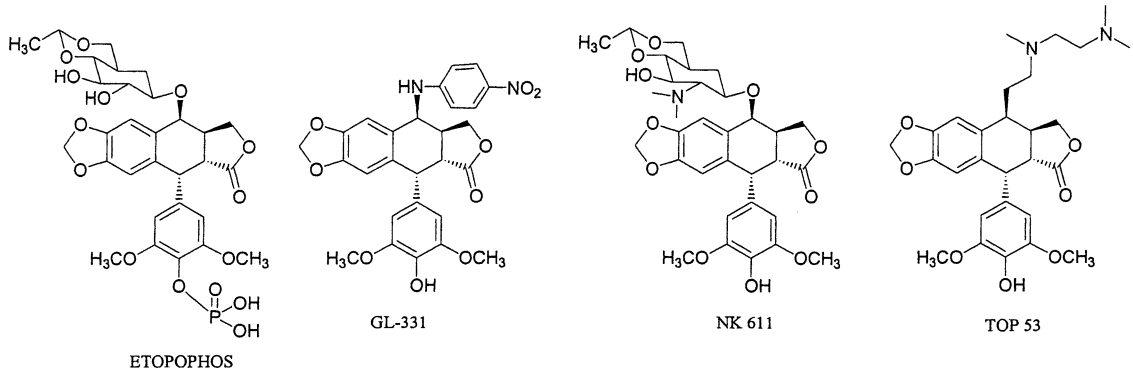


Figure 3. Representative podophyllotoxin analogs.

mediated cellular double-strand DNA breaks (Ross et al., 1984) by stabilizing the DNA-enzyme cleavable complex and inhibiting the strand-rejoining activity of topo II (Berger and Wang, 1996). This mechanism is shared by etoposide, teniposide, and many other 4′-demethylepipodophyllotoxins. The structural preferences of topo II inhibitors over antimicrotubule agents were roughly identified as: 1) demethylation at 4′ position, 2) β-configuration at 4 position, 3) bulky substitution at 4 position (Liu et al., 1989; Chang et al., 1991). Due to the severe toxic effects of antimicrotubule agents acting on the colchicine-binding site, the topo II inhibitory agents are of more clinical relevance and research interest.

Other mechanisms, including metabolic activation of the dimethoxyphenol ring E through the catechol-orthoquinone equilibrium (van Maanen et al., 1987, 1988; Sinha and Trush, 1983) and induction of apoptosis via the abnormal activation of protein tyrosine phosphatase (PTP), cell-division-cycle (CDC) 2 kinase, and CDC 25 phosphatase (Huang et al., 1996, 1997) have also been proposed for the antitumor activities of podophyllotoxin analogs.

Molecular modification and structure-activity relationship

The therapeutic application of etoposide and teniposide is often hindered by problems of aquired drug resistance, poor water solubility, and metabolic inactivation. To circumvent these problems, the synthesis and biological evaluation of podophyllotoxin analogs, particularly C_4 modified analogs, have been a primary research focus of our laboratory as well as others for many years. As highlights of such global efforts,

several synthetic analogs, Etopophos (Bristol-Myers), GL-331 (NPL at UNC), NK 611 (Nippon-Kayaku), and TOP 53 (Taiho) (Figure 3), have been produced as either clinical drugs or novel chemotherapeutic candidates for various cancers.

The molecular modification and structure-activity relationships (SAR) studies on this compound class have been thoroughly discussed in previous reviews (Zhang and Lee, 1994; Gordaliza et al., 2000a). Briefly, structural features critical for the topo II inhibition have been revealed as follows (Moraes et al., 2002): 1) the 4β-configuration is essential with variable substituents accommodated at C_4; 2) the 4$'$-hydroxy is crucial; 3) a *trans*-lactone with 2α, 3β configuration is very important; 4) the dioxolane A ring is optimal; and 5) free rotation of ring E is required.

The various projects performed in our laboratory and the four representative analogs shown in Figure 3 are discussed below to illustrate the relevance of lignans in cancer treatment and the process of drug discovery and development.

Novel podophyllotoxin analogs synthesized in NPL as antitumor agents. Because topo II inhibition is the molecular mechanism of clinical relevance, a mechanistic approach targeting the topo II enzyme should be applied to identify useful analogs. In 1989, our group first introduced two complementary assays, *in vitro* human DNA topo II inhibition and cellular protein-DNA complex formation, which were coupled with a cytotoxicity assay in KB cells, for evaluation of newly prepared analogs (Lee et al., 1989).

A. 4-amino-epipodophyllotoxin derivatives including GL-331

The development of etoposide and teniposide suggested that bulky groups could be accommodated in the C-4 position. To improve bioavailability, the nitrogen atom, a bioisostere of oxygen, was introduced at this position. The amino functionality affords the possibility of salt formation, thus increasing water solubility. Starting from podophyllotoxin, several series of 4-alkylamino and 4-arylamino epipodophyllotoxin analogs were prepared and evaluated (Lee et al., 1989, 1990; Wang et al., 1990).

Compared with etoposide, several compounds showed similar or increased percentage inhibition of topo II activity and percentage cellular protein-DNA complex formation (Table 1) (Chang et al., 1991).

Most notably, these derivatives retained cytotoxicity against drug-resistant tumor cell lines (Table 2), which implied significant therapeutic benefits against cancers that had developed resistance to various drugs, including etoposide. GL-331 (Wang et al., 1990), which contains a *p*-nitroanilino moiety at the 4β position of **3**, was selected as the optimal drug candidate and brought into clinical trials.

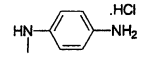
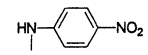
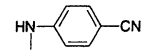
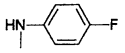
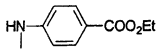
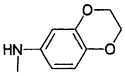
As the number of podophyllotoxin derivatives increases, formulation of a useful SAR becomes indispensable to rationally guide further synthetic efforts. A composite pharmacophore model was derived from superimposition of several topo II inhibitors of the epipodophyllotoxin, anthracycline, and aminoacridine classes (MacDonald et al., 1991), which suggested that considerable structural diversity could be well accommodated at the C_4 position of **3**. Comparative molecular field analysis (CoMFA) models generated by us (Cho et al., 1996b and Xiao et al., 2002) coincide with MacDonald's model. The steric and electrostatic contour plots of the CoMFA models indicated that bulky and electron-withdrawing substituents at the *para*-position of the 4β-anilino moiety would enhance the topo II inhibitory activity of this compound class. A *k* Nearest Neighbor (*k*NN) quantitative structure-activity relationships (QSAR) model has been recently built to rationalize the design of novel C_4 modified analogs as potent human topo II inhibitors (Xiao et al., 2002).

Based on the newly available models, a series of 4β-[(4$''$-benzamido)-amino]-DMEP derivatives were designed and synthesized. In these compounds, the 4β-[(4$''$-benzamido)-amino] moiety was included to retain the optimal activity and drug-resistance profiles of 4β-arylamino epipodophyllotoxins, while protected amino acids were introduced as a handle to modulate the water- solubility, and presumably, the bioavailability of this compound class. As predicted, most of the compounds exhibited the same superior drug-resistance profiles of GL-331. Four compounds showed comparable or even superior activity profiles to GL-331 (Xiao et al., 2004a,b, unpublished data). Further analog synthesis following this molecular design is currently ongoing.

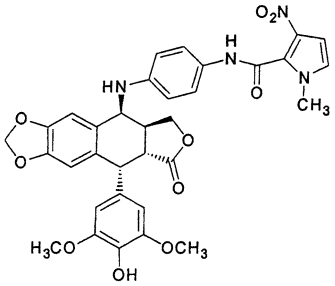
B. Etoposide analogs with minor groove binding enhancement

The contour plots of the CoMFA model (Cho et al., 1996b) also suggest that the steric and electronic fields of the DMEP derivatives are compatible with the stereochemical properties of the DNA backbone

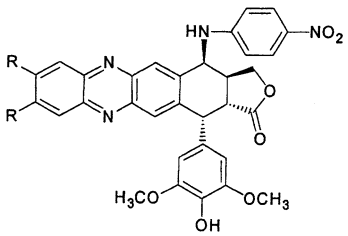
Table 1. Mechanstic screening of selected 4-arylamino epipodophyllotoxin analogs.

Compound R	$ID_{50}(\mu M)$ Tubulin polymerization	% Inhibition of tubulin at 100 μM	% Protein-linked DNA breaks	$IC_{50}(\mu M)$ for maximal DNA breaks
1	0.5	100	ND[a]	ND[a]
3	>100	0	100	10
 **(6)**	10	88	100	2
 **GL-331)**	> 100	35	140	2
 **(7)**	>100	34	125	6
 **(8)**	50	60	141	5
 **(9)**	100	50	131	5
 **(10)**	5	86	110	6

[a]ND: Not determined.



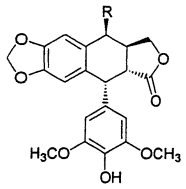
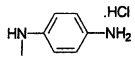
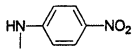
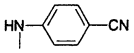
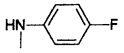
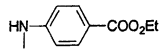
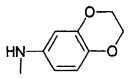
11



12 R = H
13 R = Cl
14 R = -CH=CH-CH=CH-

and the variable region at C_4 may be responsible for drug interaction with the DNA minor groove. Thus, introduction of a minor groove binding moiety at C_4 might increase topo II inhibition. The cytotoxic polypeptide netropsin contains functional groups with minor groove binding ability, and these structural components were linked to a *p*-aminoanilino epipodophyllotoxin through an amide bond (Ji et al., 1997). Among the derivatives thus produced, compound **11** and its HCl salt were found to exhibit potent cytotoxicity against several cancer cell lines (with average log GI_{50} values of -6.91, -7.0, and -5.01 for **11**, its HCl salt, and etoposide, respectively). These two compounds were extremely active against MOLT-4 leukemia and MCF-7 breast cancer cell lines with log GI_{50} values less than -8. Compound **11** showed superior inhibitory activity against DNA topoisomerase II compared to that of etoposide, displaying increased

Table 2 Cytotoxicity of selected 4-arylamino epipodophyllotoxin analogs

	Compound *R*	*$ID_{50}(\mu M)$* *KB ATCC*	*KB 1Ca*	*KB 7d*	*KB 7d^r*	*KB 50b*
	**3**	0.60	34.8	77.5	4.5	28.7
	 **(6)**	0.59	3.5	7.6	0.45	22.0
	 **(GL-331))**	0.49	6.1	7.7	0.53	3.0
	 **(7)**	0.61	2.7	5.0	0.98	4.0
	 **(8)**	0.67	4.0	8.3	0.69	7.2
	 **(9)**	0.84	2.6	7.0	0.30	3.3
	 **(10)**	0.68	0.5	1.0	0.70	1.6

*ATCC, American Type Culture Collection; KB 1Ca, KB 7d, **1**-resistant cell line with decreased cellular uptake of **1** and decreased expression of topo II; KB 7d^r, revertant cell line of KB 7d, with cellular uptake of **1** restored, but less topo II than in w.t. KB; KB 50b, vincristine-resistant cell line with overexpression of MDR1.

topo II inhibitory activity ($IC_{100} = 12.5\ \mu M$; for etoposide, $IC_{100} = 100\ \mu M$) and cellular protein DNA complex formation (225% at 2.5 μM).

C. Podophenazine derivatives as novel topo II inhibitors

In MacDonald's model, an intercalation or "intercalation-like" domain overlaps with the methylenedioxy ring of DMEP derivatives (MacDonald et al., 1991). Furthermore, CoMFA steric contour plots also show a sterically favorable area in the same region (Cho et al., 1996b). These studies thus suggest that the methylenedioxy ring is another logical area of synthetic modification. By synthesizing podophenazine derivatives (**12**–**14**), the methylenedioxy ring of GL-331 was replaced by a quinoxaline heteroaromatic ring system, which extends the planar aromatic area further into the "intercalation" domain of MacDonald's model. Compared with **3**, compounds **12** and **13** showed comparable or greater cytotoxicity against KB and **3**-resistant KB-7d cells. Interestingly, these compounds did not stimulate DNA breakage and, thus, do not inhibit topo II in the same manner as does **3** (Cho et al., 1996a).

D. γ-Lactone Ring-Modified 4-Amino Etoposide Analogs

Metabolism of etoposide analogs occurs by either epimerization to the *cis*-picro-lactone or by hydrolysis to the *cis*- and *trans*-hydroxy acids (Figure 4); all three compounds are inactive. To ameliorate the metabolic inactivation, we replaced the lactone carbonyl with a methylene group to generate D-ring tetrahydrofuran derivatives (**15, 16**) and substituted the 2-hydrogen atom with fluoro to provide 2-fluoro derivatives (**17–21**). Compounds **15** and **16** were equally active as **3** in topo II inhibition ($ID_{50} = 50\ \mu M$) and more potent than **3** in cellular protein-DNA complex formation (125, 139, and 100% at 20 μM for **15**, **16**, and **3**, respectively) (Zhou et al., 1994). Compounds **17–21** were moderately active against several cancer cell lines, but they were much less active than the cor-

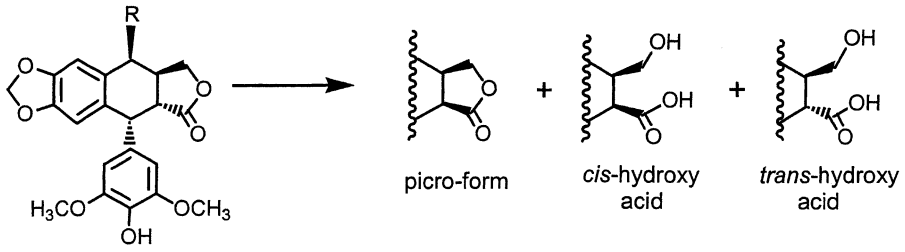
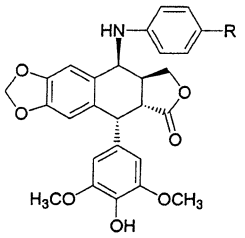
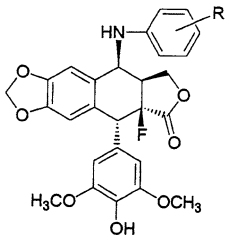


Figure 4. Metabolism of Etoposide Analogs.



15 R = H
16 R = F



17 R = *p*-F
18 R = *p*-CN
19 R = *p*-NO_2
20 R = 3,4-OCH_2CH_2CO-
21 R = *p*-NH_2

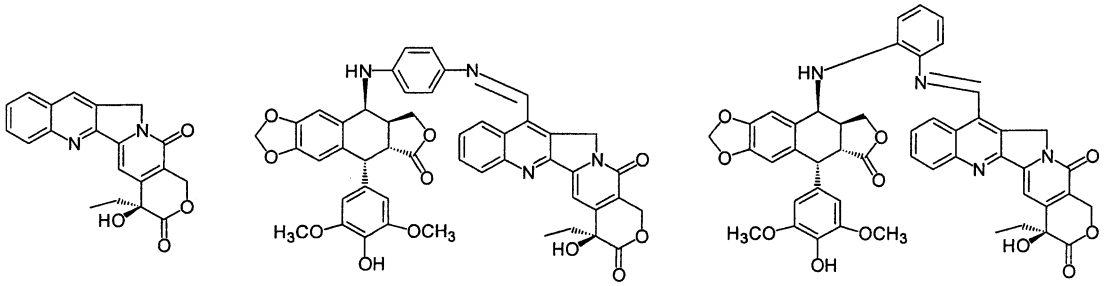
responding nonfluorinated analogs (VanVliet et al., 2001).

E. Dual topo I and topo II inhibitors

Although most topoisomerase inhibitors specifically target either topo I (e.g. camptothecin, **22**) or topo II (e.g. etoposide, **3**), other compounds, such as the 7-*H*-benzopyrido[4,3-*b*]indole derivative inotoplicine, simultaneously inhibit both topo I and topo II and, thus, may circumvent topoisomerase-mediated drug-resistance mechanisms. We hoped to generate dual inhibitors of both enzymes by chemically linking a *p*- or *o*-aminoanilino substituted epipodophyllotoxin with 4-formyl camptothecin through an imine bond to give hybrid lignans. Indeed, these compounds, **23** and **24** (Bastow et al., 1997), did stimulate DNA cleavable complex formation with both topo I and II. Both compounds were about half as active as **22** in the former assay, and **24**, but not **23**, was as active as **3** in the latter assay. Compared with **3** and GL-331 (**6**), **23** and **24** were 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 (data not shown). More importantly, in KB and drug-resistant KB-variants, **23** and **24** showed a much lower decrease in cytotoxicity (ca. twofold and sixfold) than did **3** (80-fold) in a **3**-resistant cell line (KB-7D) or **22** (30-fold) in a **22**-resistant cell line (KB-CPT). Both conjugate compounds also showed a lower decrease in a vincristine-resistant cell line (KB-VCR) than did **3** (Table 3). When given *i.p.* to nude mice, **24** showed low *in vivo* toxicity. In general, conjugation resulted in cleavable complex-forming dual topoisomerase inhibitors with improved cytotoxic activity against drug-resistant cells, and this area of research is being further explored in our laboratory.

To develop epipodophyllotoxin analogs with multiple antitumor mechanisms and improved drug-resistant profiles, we prepared and evaluated five taxoid-epipodophyllotoxin conjugates as novel cytotoxic agents (Shi et al., 2001). Some of the conjugates showed comparable or better activity than epipodophyllotoxin derivatives in most tumor cell lines and topo II inhibition assays. Although the differences between the cytotoxic profiles of the conjugates and the parent compounds suggested that novel cell killing mechanisms might be involved, additional investigations are necessary to fully access the activity profiles and mechanism of action of these newly synthesized conjugates.

Table 3 Cytotoxicity of 23 and 24 against the KB cell line and resistant variants.



22 **23** **24**

	IC_{50} (nM)[a]			
Compound	*KB*	*KB-CPT*	*KB-7D*	*KB-VCR*
3	425	500	32,000	2,000
22	9	292	14	18
23	33	182	70	70
24	33	198	70	100

[a]IC_{50} values were determined after 72 h of culturing with continuous exposure to test compounds.

Representative podophylltoxin analogs The clinical efficacy of etoposide and teniposide has stimulated a wide range of research programs geared to overcome the problems of poor water solubility or drug resistance. These programs have resulted in the discovery and development of several novel podophyllotoxin analogs with improved pharmacological and pharmacokinetic profiles.

Etopophos

The poor water solubility of **3** and **4** limits the possible routes of drug administration and increases the difficulty of drug formulation. Large doses of etoposide may require administration of significant fluid volumes, which increases the risk of heart failure in some patients and presents an obstacle to rapid administration as well as long-term infusional administration regimens. Hypersensitivity reactions and hypotension may also occur, probably due to the vehicles needed as solubilizers (Hande, 1998).

Extensive molecular modification of etoposide, especially modification of the aromatic E ring by introducing a range of substituents at position 4′, was carried out at the Bristol Myers company. As a result, Etopophos ™ (etoposide phosphate), a water-soluble prodrug of etoposide, was developed (Saulnier et al., 1989) and approved for intravenous use by the FDA in 1996. Etopophos is less toxic and more active than etoposide in *in vivo* tumor models (Saulnier et al., 1989) and is soluble in water at concentrations up to 20 mg/ml (Long and Casazza, 1994). It can be efficiently converted *in vivo* by endogenous phosphatase to the active drug etoposide, and exhibits pharmacological and pharmacokinetic profiles similar to those of etoposide (Budman, 1996; Greco and Hainsworth, 1996). The improved drug administration ability makes Etopophos preferable to etoposide for routine clinical use.

B. NK 611

To tackle the problems of poor water solubility and bioavailability, another etoposide derivative NK 611, which has a dimethylamino side chain instead of a hydroxyl group at the 2″ position of the ethylidene glucoside moiety, was developed (Saito et al., 1986). The introduction of an amine made it possible to improve water solubility by forming salts. NK 611 showed promising antitumor activity against human tumor xenografts with a similar potency to that of etoposide (Hanauske et al., 1994); however, it proved to be a more potent topoisomerase II inhibitor and was more cytotoxic against a variety of human cancer lines including lung, gastrointestinal, ovarian, testicular, breast, head and neck, and leukemias (Pagani et al., 1996). Clinical tests of intravenous and oral formulations of NK 611 suggest that the compound

has better bioavailability than etoposide (Mross et al., 1996; Pagani et al., 1996).

C. GL-331

It was previously believed that the presence of a glucoside group at 4β is necessary for the topo II inhibitory activity; however, the introduction of a *p*-nitro anilino group at the 4β position led to the discovery of a novel, potent topo II inhibitor, GL-331, which is currently in phase II clinical trials against various cancers (Lee KH, 1999). Like **3**, GL-331 functions as a topo II inhibitor, causing DNA double-strand breakage and G2-phase arrest (Lee et al., 1990). GL-331 and **3** also cause apoptotic cell death by inhibiting protein tyrosine kinase activity (both compounds) and by stimulating protein tyrosine phosphatase activity and apoptotic DNA formation (GL-331) (Huang et al., 1996; 1997). Compared with **3**, GL-331 is more active both *in vitro* and *in vivo,* and more remarkably, it overcomes multidrug resistance in many cancer cell lines (KB/VP-16, KB/VCR, P388/ADR, MCF-7/ADR, L1210/ADR, HL60/ADR, and HL60/VCR) (Liu et al., 1993; Lee KH, 1999). This compound is a more potent inhibitor of proliferation of refractory cancer cell lines than **3** (Huang et al., 1999). Initial results from Phase I clinical trials in four tumor types (nonsmall and small cell lung, colon, and head/neck cancers) showed marked antitumor efficacy. Side effects were minimal with cytopenias being the major toxicity. Maximum tolerated dose (MTD) was declared at 300 mg/m^2 (Lee KH, 1999).

Formulated GL-331 shows desirable stability and biocompatability, and similar pharmacokinetic profiles to those of **3** (Liu et al., 1993; Lee KH, 1999).

D. TOP 53

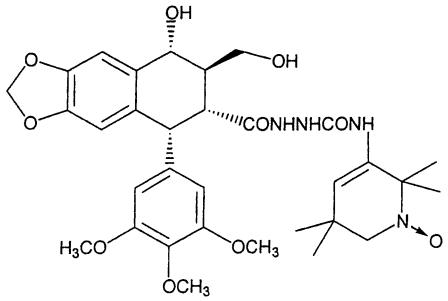
TOP 53 was selected from a series of 4β-alkylated etoposide analogs, in which the glycoside group of etoposide was replaced with a carbon chain containing hydroxyl, amino or amido groups (Terada et al., 1993). As compared to **3**, TOP 53 was a more potent inhibitor of topo II; it was twice as active as **3** or as effective as **3** at 5 to 6 times lower concentration (Utsugi et al., 1996). It showed nearly wild-type potency against a mutant yeast type II enzyme highly resistant to **3** (Byl et al., 2001), implying therapeutic potential for drug-resistant cancers. TOP 53 exhibited strong activity against a wide variety of tumor cells, with especially high activity against non-small cell lung cancer in both tumor cells and animal tumor models (Byl et al., 2001). It was also found particularly distributed in the lung, which suggested that it might be exceptionally suitable for treating lung cancer and its metastases (Imbert, 1998). TOP 53 is currently in Phase I clinical trials (Moraes et al., 2002).

Clinical application of etoposide and teniposide

Etoposide shows a broad spectrum of antitumor activity against a variety of cancers. It is effective against ascitic tumors, testicular tumors, ovarian and gestational carcinomas, different types of lung cancer (small-cell, squamous, adenocarcinoma, Lewis carcinoma), leukemias (monocytic and refractory), malignant and recurrent lymphomas, urogenital tumors, sarcomas, melanomas, and experimentally-induced colon cancer (O'Dwyer et al., 1985; Fleming et al, 1989; Utsugi et al., 1996).

Etoposide is widely used as an anticancer drug, especially in combination chemotherapeutic regimens. The combination of etoposide with *cis*-platin, a most effective protocol, shows a highly productive first-line therapeutic synergism in small-cell cancer and it is used as a second line or salvage therapy for CAV (Cyclophosphamide, Adriamycin and Vincristine) resistant or failure small-cell lung cancer (O'Dwyer et al., 1985; Ayres and Loike, 1990). It is also effective for the treatment of testicular cancer (Hainswirth et al., 1985) and non-small-cell lung cancer (Finkelstein et al., 1986). Etoposide is also an effective agent for incorporation into other multi-drug treatment protocols for the treatment of non-Hodgkin lymphomas resistant to other agents, different types of lymphomas, refractory childhood leukemia, hepatocellular metastasis, refractory acute lymphoblastic leukemia, and other types of cancers (Ayres and Loike, 1990; Gordaliza et al., 2000a). As a water-soluble prodrug of etoposide, etopophos exhibits pharmacological and pharmacokinetic profiles similar to those of etoposide and is preferable for routine clinical use, especially for intravenous administration.

Teniposide, which is more potent than etoposide, shows good antitumor activity against different types of cancer (Fleming et al., 1989; Ayres and Loike, 1990), including lymphoblastic, acute lymphocytic leukemia and other experimentally-induced leukemias, infantile non-Hodgkin lymphoblastic lymphoma, multiple myeloma, ascitic tumours, malignant brain tumours, colo-rectal and refractory or recurrent testicular carcinomas, and small-cell and non-small-cell lung cancers. Combination chemotherapy incorporating teniposide has also been applied to various cancers. It is used in combination with *cis*-platin



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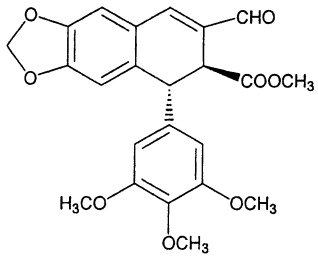

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against neuroblastoma (McWilliams et al., 1995), with cytarabine (ara-C) against acute lymphoblastic leukemia, and with carboplatin in small-cell lung cancer (Michel et al., 1994).

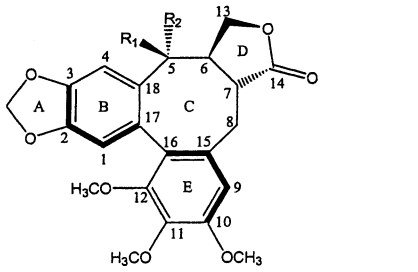
Like most other topoisomerase II inhibitors, etoposide and teniposide may induce myeloid leukemia (Pui et al., 1995). Other adverse effects of these drugs, including anemia, catharsis, and alopecia, (Gordaliza et al., 2000a) are due to failure to distinguish tumur cells from other cells in the active phase of division.

Aryltetralin lignans

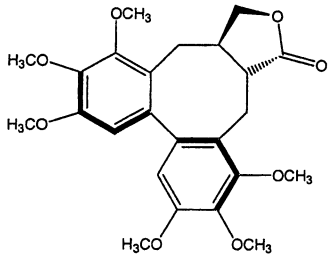
Although an intact lactone D ring seemed to be important for the topo II inhibitory activity of podophyllotoxin analogs, three aryltetralin lignans without the lactone moiety, (+)-dimethylisolariciresinol-2-α-xylose, picropodophyllic acid, and podophyllic acid ethylhydrazide, had been reported as antitumor lignans before 1984 (MacRae and Towers, 1984). Later, a D ring modification of podophyllotoxin produced GP-11 (**25**), which was almost equipotent with etoposide (Wang et al., 1993). More recently, several cyclolignans lacking the lactone ring were prepared from podophyllotoxin or deoxypodophyllotoxin and evaluated for cytotoxic activity. Podophyllic acid methyl ether exhibited high potency against breast, ovarian, prostate, colon, renal, and lung cancer cell lines with 50% growth inhibition (GI_{50}) ranging from 10 to 60 nM (Subrahmanyam et al., 1998). Methyl 9-deoxy-9-oxo-α-apopicropodophyllate (**26**) and its 2,2,2-trifluoroethyl-hydrazone and phenylhydrazone derivatives showed highly selective cytotoxicity against the HT-29 human colon carcinoma (IC_{50} = 12, 48, and 39 ng/mL, respectively, Gordaliza et al., 1997). Several related aldehyde analogs with different configurations were synthesized and evaluated for cytotoxic activity. All of them lacked the lactone ring but retained their cytotoxicity at or under the μm level (Gordaliza et al., 2000b). The above results of antitumor aryltetralin lignans suggested that dramatic modification of the D ring might provide clinically useful agents with altered pharmacological profiles. Molecular targets other than topo II are likely involved in the antitumor activity of these lignans.

Steganacin analogs

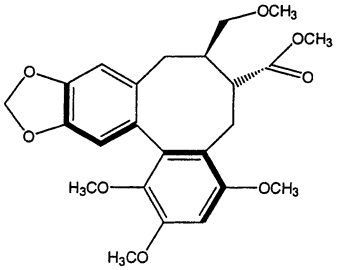
Steganacin **27** and steganangin **28** are cytotoxic lignan lactones first isolated from the alcoholic extract of *Steganataenia araliacea* Hochst (Umbelliferae), which showed significant antitumor activity against P-388 leukemia in mice and the human nasopharynx carcinoma KB cell line *in vitro* (Kupchan et al., 1973). These compounds are dibenzocyclooctadiene lignans structurally similar to podophyllotoxin. Like podophyllotoxin, steganacin also inhibited microtubule assembly via binding to the colchicine-binding site of tubulin (Schiff, 1978). Most natural steganacin analogs possess the same aR, 5R, 6R, 7R configuration as (-)-steganacin and the spatial arrangement of the two aryls (rings B and E) in this configuration is similar to that in podophyllotoxin and colchicine. Structural features critical for the cytotoxicity of stegnanacin analogs were identified as follows: 1) the R configured chiral axis is essential; 2) C_5 α-configuration is important and deoxygenation or bulky substitution at C_5 decreases activity significantly; 3) an intact lactone D ring is crucial; and 4) demethylation of either C_{10} or C_{11} in ring E also results in loss of activity (Sackett, 1993; Zavala, F. 1980; Tomioka et al., 1991). Steganacin (**27**), steganangin (**28**), epi-steganangin (**29**), steganolide A (**30**), and steganoate B (**31**) showed cytotoxic activity against a panel of 11 human tumor cell lines with ED_{50} values between 0.4–



	R_1	R_2
27 steganacin	H	OAc
28 steganangin	H	$-O_2C$
29 episteganangin	$-O_2C$	H



30 steganolide A



31 steganoate B

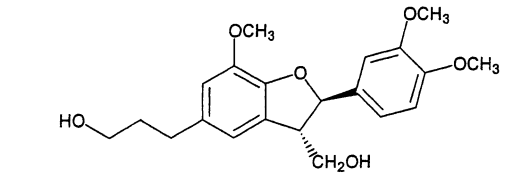
17.3 μg/ml (Wickramaratne et al., 1993). In spite of their cytotoxicity, no steganacin derivative has found medical application.

Due to the close structural and SAR similarities between steganacin and podophyllotoxin analogs, it is reasonable to postulate that bulky 5β-substitution and demethylation at C_{10} and C_{11} might produce steganacin analogs targeting topo II. It should also be noted that the restriction of E ring free rotation by the octadiene ring might contribute to the relatively low potency of steganacin analogs.

Dihydrobenzofuran lignans

Dihydrobenzofuran lignans belong to the neolignan class, in which the two C_6-C_3 units are linked together by a bond other than Cβ-Cβ'. The investigation of dihydrobenzofuran lignans derived from the isolation of 3′, 4-di-*O*-methylcedrusin (**32**), which was a minor lignan of 'Dragon's blood' (*Croton* spp., Euphorbiaceae) and was found to be an inhibitor of cell proliferation (Pieters et al., 1993). A series of dihydrobenzofuran lignans were synthesized and evaluated in a human disease-oriented tumor cell line screening panel *in vitro* (Pieters et al., 1999). Among these lignans, **33** showed promising antitumor activity against a variety of cancer cell lines, with an average GI_{50} value (the molar drug concentration required for 50% growth inhibition) of 0.3 μM. This compound also inhibited mitosis at micromolar concentrations in cell culture through a relatively weak interaction at the colchicine binding-site on tubulin. However, the weak antimitotic activity seemed to be insufficient to account for the cytotoxicity of compound **33**. Several dihydrobenzofuran lignans were recently tested for their antiangiogenic activity in the CAM (chorioallantoic membrane) assay and **33** exhibited pronounced antiangiogenic activity (Apers et al., 2002). For both the antimitotic and antiangiogenic activities, **33** showed a stereo-preference for the (2R, 3R) isomer. Angiogenesis is an emerging target for cancer chemotherapy, and as a novel lead for antiangiogenic agents, **33** and its analogs merit further investigation.

Additional naturally occurring dihydrobenzofuran lignans with antitumor activity have been reported recently. A novel cyclopentabenzofuran aglaiastatin (**34**), the known rocaglaol (**35**), and its related pyrimidinone isolated from *Aglaia odorata* (Meliaceae) were potent inhibitors of the growth of K-*ras*-NRK cells, with IC_{50} values of 1–10 ng/ml, induced normal morphology in K-*ras*-NRK cells at 10–30 ng/ml, and specifically inhibited protein synthesis. Aglaiastatin was the most effective and reduced the amount of Ras, probably by inhibiting its *de novo* synthesis (Ohse et al., 1996). Five rocaglaol derivatives isolated from *Aglaia elliptica* (Meliaceae) strongly inhibited the growth of 12 human cancer cells in culture with IC_{50} values of 0.8–30 ng/ml (Cui et al., 1997 and Lee SK et al., 1998). In further assays, compound **36**



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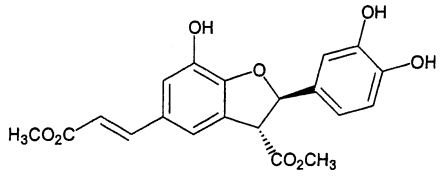

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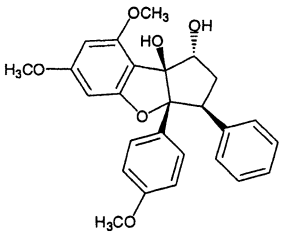

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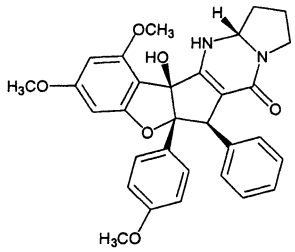

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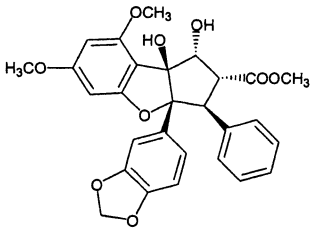

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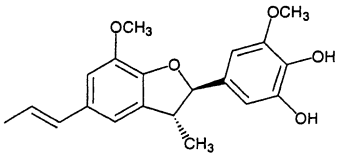

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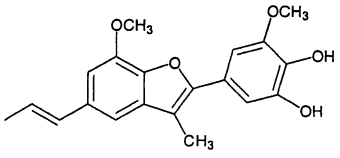

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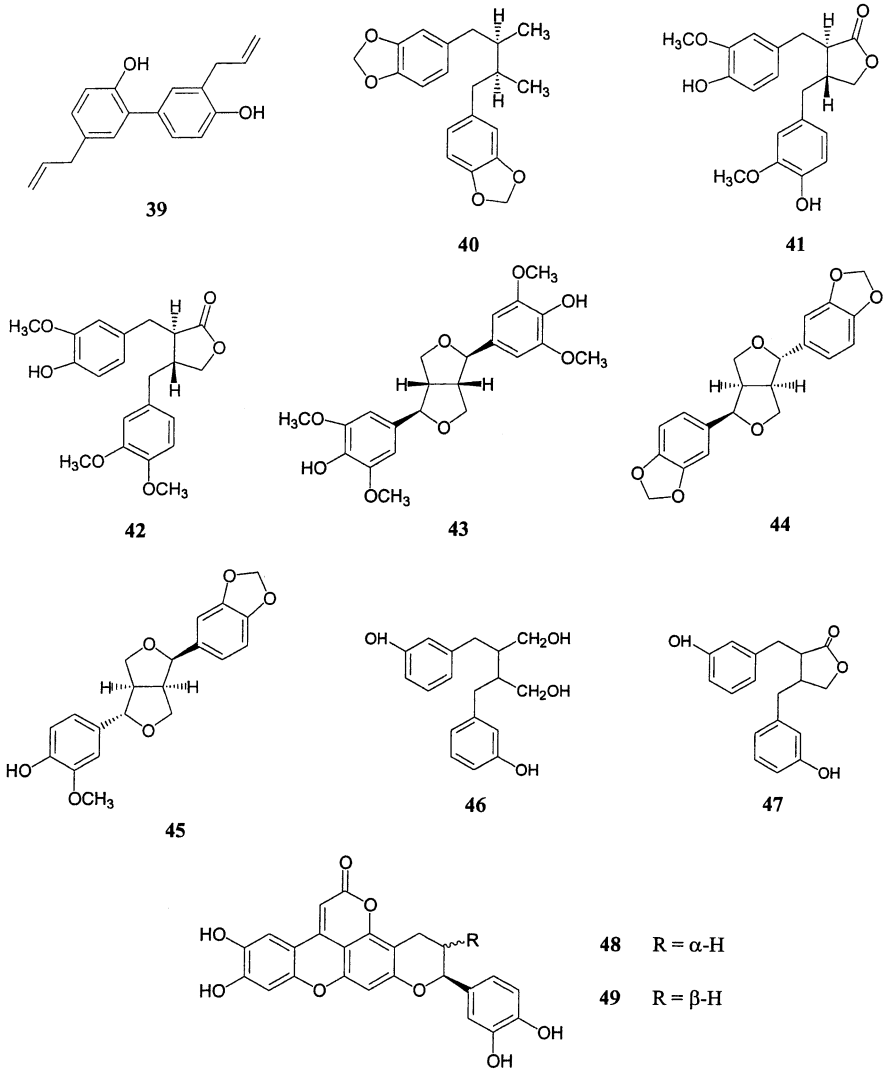
(IC_{50} = 0.9 ng/ml) induced accumulation of cells in the G1/G0 phase after 24 or 32 h of incubation and inhibited cell proliferation in a dose-dependent manner, but showed no reduction of colony formation. Compound **36** also strongly inhibited protein biosynthesis (IC_{50} = 25 ng/ml), but showed no effect on nucleic acid biosynthesis at concentrations as high as 1 μg/ml. In animal models, **36** delayed tumor growth in athymic mice without noticeable toxic effects or loss of body weight.

Seven dihydrobenzofuran or benzofuran lignans isolated from the leaves of *Persea obovatifolia* (Lauraceae) were evaluated as cytotoxic agents against several tumor cell lines (Tsai et al., 1996, 1998). All of these lignans showed marked cytotoxicity against the tested tumor cell lines with obovatifol (**37**), the most active, showing ED_{50} values close to those of the reference drug, mithramycin, against the KB16 cell line. Acetylation of obovatifol (**37**) and obovaten (**38**) gave two diacetates even more potent than the reference drug against the KB16 cell line (ED_{50} = 0.075 and 0.049 μg/ml, respectively).

Other antitumor lignans

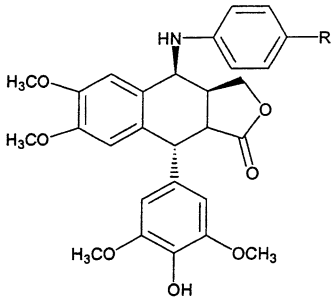
Other lignans merit inclusion in this discussion. Honokiol (**39**), machilin A (**40**), matairesinol (**41**), and arctigenin (**42**) inhibited the growth of the human promyelocytic leukemic cell line HL-60 (IC_{50} values ranging from 10–940 ng/ml) with little or no cytotoxicity against the tested cells (LC_{50} > 2.9 μg/ml), probably through a non-toxic mechanism interfering with the DNA, RNA, or protein synthesis of the leukemic cells (Hirano et al., 1994). Syringaresinol (**43**) from *Annona montana* (Annonaceae) and its acetate showed significant cytotoxicity (ED_{50} values of 0.67 and 3.78 μg/ml, respectively) against P-388 cells (Wu et al., 1995). Asarinin (**44**) and xanthoxylol (**45**) from *Asiasarum heterotropoides* var. *mandshuricum* (Aristolochiaceae) exhibited remarkable inhibitory effects in a two-stage carcinogenesis test of pulmonary tumors on mouse skin (Takasaki et al., 1997). Various mammalian lignans, such as enterodiol (**46**) and enterolactone (**47**), have been widely studied as chemopreventive agents and likely exert cancer-protective effects through antagonism of estrogen metabolism, an-



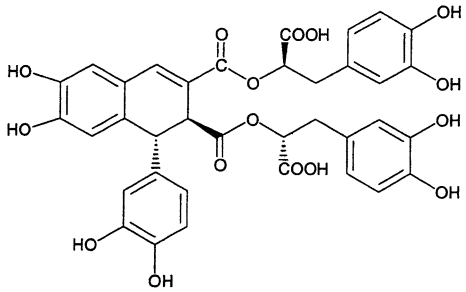
tioxidant activity, and modulatory effects on key control points of the cell cycle (Rickard and Thompson, 1997). Two flavanolignans, mururins A and B (**48** and **49**) from *Brosimum acutifolium* Huber, showed 3% and 63% inhibition of protein kinase A and 58% and 38% inhibition of protein kinase C, respectively, at 20 μM (Takashima et al., 2002). Antitumor lignans reported before 1999 have been covered by previous reviews (MacRae and Towers, 1984; Konuklugil, 1994; Ward, 1997; Ríos et al., 2002). These novel lignans with promising antitumor activity provide new leads for anticancer drug development and broaden the scope of current investigations.

Antiviral lignans

Investigation of the antiviral activity of lignans began with the finding that an alcoholic extract of *Podophyllum peltatum* could be used as a topical treatment for venereal warts (*Condyloma acuminatum*), an ailment caused by a papilloma virus (Kaplan, 1942). To date, lignans belonging to the aryltetralin, arylnaphthalene, dibenzocyclooctadiene dibenzylbutane and dibenzylbutyrolactone skeletal types have been reported to show antiviral activity. The mechanisms of action involve inhibition of tubulin polymerization, reverse transcriptase, integrase, and topoisomerases. Antiviral lignans have been previously covered by several excellent reviews (MacRae and Towers, 1984; Charlton



50 R = CN
51 R = CO_2Et
52 R = NO_2
53 R = F



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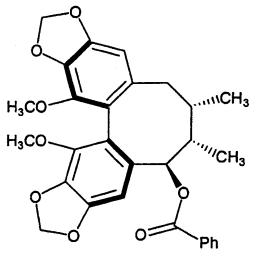
1998; Ríos et al., 2002). In general, the antiviral effects of lignans are not strong. Therefore, Charlton concluded that commercial application of antiviral lignans might be limited to podophyllotoxin, which is used topically to treat various warts caused by human papilloma virus (HPV). Because acquired immune deficiency syndrome (AIDS), which is caused by the human immunodefiency virus (HIV), is currently the viral infection of most research interest, we will only highlight representative anti-HIV lignans discovered in Dr Lee's NPL.

Aryltetralin lignans

Podophyllotoxin (**1**) is by far the most relevant and best known antiviral lignan. Podophyllotoxin and its natural and synthetic analogs have been assayed against various viruses, including HIV. Notably, four analogs (**50–53**) with the methylenedioxy A-ring opened and methylated and the 4′-position demethylated showed significant inhibition of HIV replication with EC_{50} values less than 0.001 μm and therapeutic indices greater than 120 (Lee et al., 1997). Naturally occurring anti-HIV aryltetralin lignans have also been found. Three sodium and potassium salts of rabdosiin (**54**) and its 1(S), 2(R) isomer isolated from *Arnebia euchroma* (Boraginaceae) showed potent anti-HIV activity with EC_{50} values of 2.8, 4.0, and 1.5 μg/ml and therapeutic indices of 19.6, 12.5, and 33.3, respectively (Kashiwada et al., 1995a). Rabdosiin and its isomer were later reported to be non-selective inhibitors of mammalian DNA topoisomerases, which suggested that the anti-HIV activity might be due to topoisomerase inhibitory activity (Kashiwada et al., 1995b).

Dibenzocyclooctadiene lignans

The ethanol extract of the stems of *Kadsura interior* showed significant inhibitory activity *in vitro* against HIV replication in H9 lymphocytes. Bioactivity-directed fractionation and isolation identified interiotherin A (**55**) and schisantherin D (**56**) as the active principles with EC_{50} values of 3.1 and 0.5, and therapeutic index values of 13.2 and 50.6, respectively (Chen et al., 1996). In further studies, seven of the 12 known lignans isolated from the same species were active as anti-HIV agents, with gomisin-G (**57**) being the most potent (EC_{50} and therapeutic index values of 6 ng/ml and 300, respectively). Schisantherin D (**56**), schisantherin C (**58**), and kadsuranin (**59**) also showed good activity, with EC_{50} values of 0.5, 0.8, and 1.2 μg/ml and therapeutic index values of 110, 56, and 33.3, respectively (Chen et al., 1997). The results with these natural lignans suggested that 9-benzoyl and 8-hydroxy substituents might enhance anti-HIV activity. To determine the optimal pattern(s) of biphenyl substitution and the role of the cyclooctane ring, a series of hexahydroxybiphenyl derivatives were synthesized and evaluated for anti-HIV activity (Xie et al., 1995). Two brominated derivatives, **60** and **61**, demonstrated potent anti-HIV activity with EC_{50} values of 0.52 and 0.23 μg/ml and therapeutic index values of > 190 and > 480, respectively. These results suggested that the relative position and types rather than the number of the substituents on the phenyl rings of both the natural lignans and the synthetic biphenyls were of primary importance, while the cyclooctane ring might not be essential for anti-HIV activity. Compounds **60** and **61** also showed potent inhibitory activity against HIV-1 reverse transcriptase (RTase) in a template-primer dependent manner, which is an action mode shared



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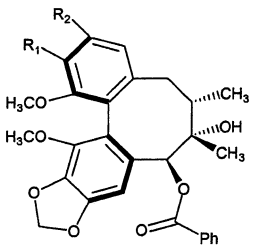
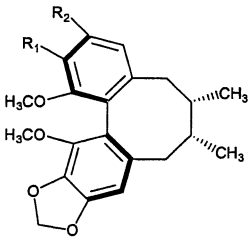
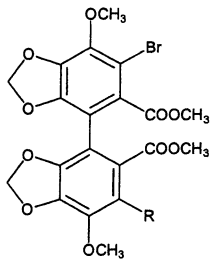


56 $R_1 + R_2 = OCH_2O$
57 $R_1, R_2 = OCH_3$

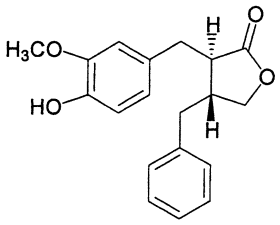


58 $R_1 + R_2 = OCH_2O$
59 $R_1, R_2 = OCH_3$



60 R = H
61 R = Br

by other non-nucleoside HIV RTase inhibitors (Tramontano and Cheng, 1992; Pauwels et al., 1990). Although the activity profile of these lignans is inferior to that of AZT, the anti-AIDS drug used clinically (EC_{50} and therapeutic index values of 2 ng/ml and 250,000, respectively), gomisins A and J were formulated for use as anti-AIDS agents by Tsumura & Co. (Jpn. Kokai Tokyo Koho JP 05, 301,816, 1993). Further structural modification of the lead compounds may produce clinically relevant compounds.

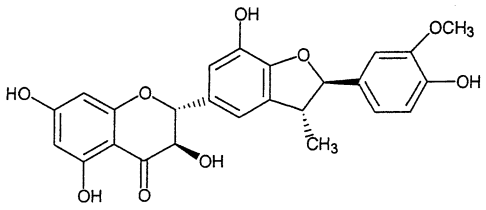


Dibenzylbutyrolactone lignans

(-)-Arctigenin (**42**) was found to reduce HIV viral protein production and inhibit viral reverse transcriptase activity as well as topoisomerase II activity (Schröder et al., 1990). Following this lead, we synthesized five (-)-arctigenin analogs and evaluated their inhibitory activity against HIV-1 replication in acutely infected H9 cells. (-)-Arctigenin and its unsubstituted benzyl derivative (**62**) showed anti-HIV replication activity with EC_{50} values of 0.16 and 2.2 μg/ml and therapeutic index values of 5 and 9.1, respectively (Yang et al., 1996). Bioassay-directed fractionation of the ethyl alcohol extract of the stems of *Symplocos setchuensis* Brand (Symplocaceae) identified matairesinol (**41**) as one of the anti-HIV principles with EC_{50} and therapeutic index of 2.0 μM and 11.0, respectively (Ishida et al., 2001). Further analog synthesis is necessary to fully access the anti-HIV activity of this skeletal type of lignan.

Hepatoprotective lignans

Lignans with protective effects on liver function have been summarized by Ríos et al. (2002) and those with clinical relevance will be highlighted here. The flavolignans of *Silybum marianum* (Compositae) fruit are antihepatotoxic agents in therapeutic use. Silandrin, silybin, silychristin, 3-deoxysilychristin, silydianin, and silymonin were tested for their CCl_4- and galactosamine-induced cytotoxicity in primary cultured rat hepatocytes (Hikino et al., 1984a). All of them reduced the cytotoxic effects of CCl_4 and galactosamine at 1 mg/ml, but only 3-deoxysilychristin (**63**) was significantly active at 0.01 mg/ml. Using the same experimental model, Hikino et al. evaluated the antihepatotoxic activity of 22 lignans isolated from *Schisandra chinensis* and *Kadsura japonica*, which are medicinal plants used for treatment of hepatitis (Hikino et al., 1984b). Wuweizisu C (**64**) and schisantherin D (**56**) showed significant activity in the CCl_4-test at 0.01 mg/ml, and deoxygomisin A, gomisin N, wuweizisu C, gomisin C, and schisantherin D were most active in the galactosamine-test at the same dose. A methylenedioxy group in the dibenzocyclooctane skeleton was suggested to be the structural feature critical for the antihepatotoxic activity of this lignan class. Mechanistic investigation of wuweizisu C and gomisin A (**65**) showed that neither compound inhibited enzymatic CCl_3 radical formation; however, both lignans inhibited CCl_4-, ADP/Fe^{3+}- and ascorbate/Fe^{2+}-induced lipid peroxidation. These results suggested that the hepatoprotective effects of the *Schisandra* and *Kadsura* lignans might occur via inhibition of lipid peroxidation at a non-enzymatic step (Kiso et al., 1985). *In*



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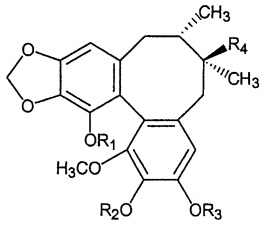


64 $R_1 = CH_3$, $R_2 + R_3 = CH_2$, $R_4 = H$
65 $R_1 = CH_3$, $R_2 = R_3 = CH_3$, $R_4 = OH$
66 $R_1 = H$, $R_2 = R_3 = CH_3$, $R_4 = H$

vivo study using induced acute hepatic failure rat models showed that when fed at 0.06% in food for four-five weeks, gomisin A increased survival rate of the experimental rats from 10% to 60–80%. No liver cell degeneration or necrosis was microscopically detectable, and both splenocyte response and productivity were significantly higher. These results suggested that gomisin A or other related lignans might be used to treat or prevent fulminant hepatitis, a poorly treatable and dangerous type of hepatitis (Mizoguchi et al., 1991). Bioassay-directed fractionation of an ethanolic extract of *Kadsura matsudai* Hayata, a Taiwanese Schizandraceae plant, identified schizanrin B (**66**) as a moderate to strong antihepatitis agent in both anti-HBsAg and anti-HBeAg assays, and schizanrins D and E and a homolignan, taiwanschirin D, were also active in the latter assay. These results suggested that the C-6 substituent in C_{18} lignans and the corresponding C-5 substituent in C_{19} homolignans could be significant for bioactivity (Li et al., 2000; Kuo et al., 2001).

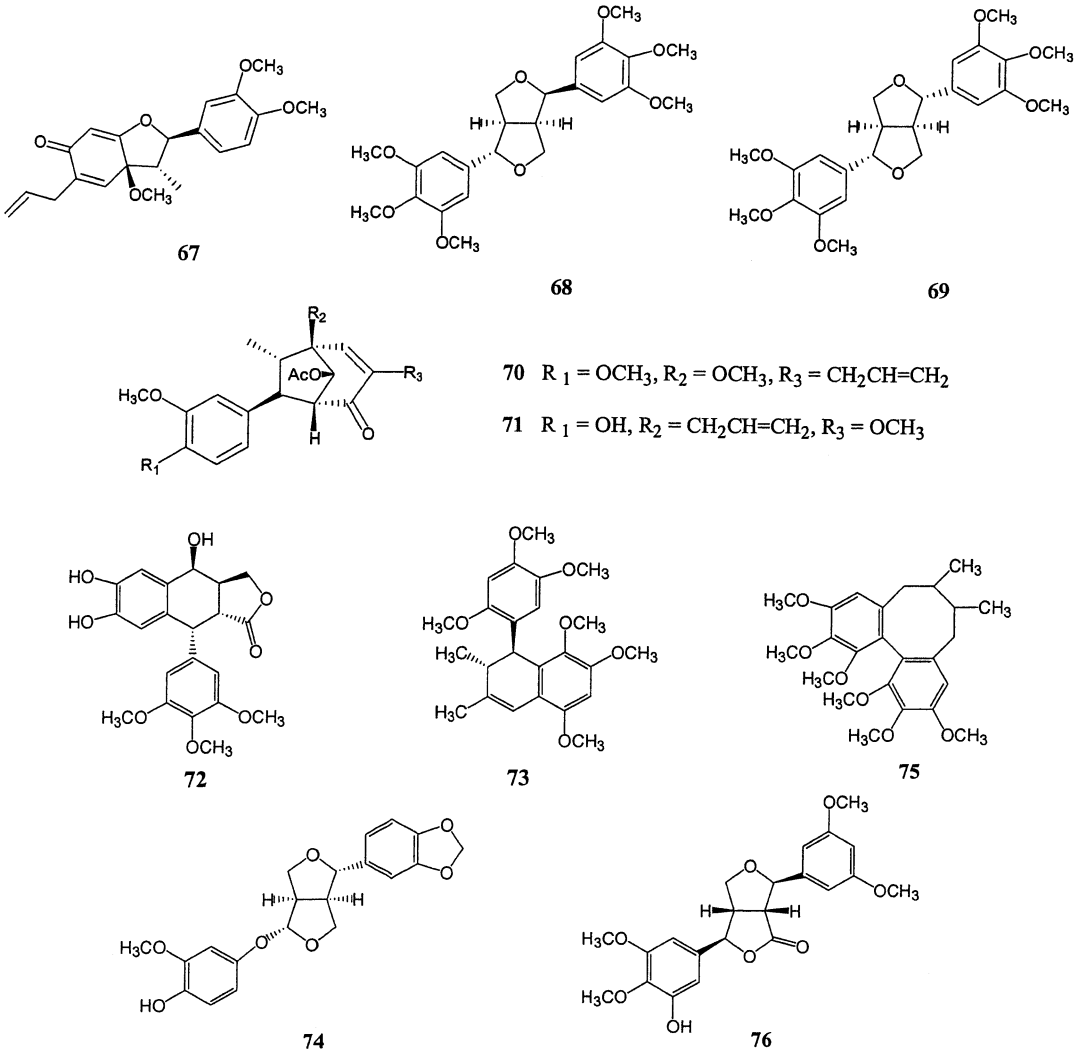
Lignans as platelet activating factor (PAF) receptor antagonists

Platelet activating factor (PAF) is a biologically active phospholipid involved in physiological and pathological processes such as asthma, allergies, inflammation, respiratory and cardiovascular events, blood coagulation, and immunity. Specific PAF antagonists may be of clinical interest in treating the above diseases. Lignans identified as PAF antagonists have been reviewed by Ríos et al. (2002) and only representative structures will be discussed here.

Kadsurenone (**67**) from *Piper futakadsura* is an inhibitor of PAF binding and of PAF-induced platelet aggregation. It did not inhibit platelet aggregation induced by ADP, collagen, arachidonic acid (AA), or thrombin even at 50 μM, but it did inhibit the platelet aggregation induced by PAF with an IC_{50} value of 3.50 μM. The dose-response curves of displacement of [^{3}H]-PAF showed that kadsurenone and other related natural and synthetic lignans competitively inhibited the PAF binding to its receptor (Shen and Hussaini, 1990; Shen, 1991). The furofuran lignans epiyangambin (**68**) and (+)-yangambin (**69**) competitively inhibited PAF-induced rabbit platelet aggregation in a dose-dependent manner with IC_{50} values of 0.61 and 1.93 μM, respectively, but they had no effect on the platelet aggregation induced by collagen, thrombin, or ADP. *In vivo* administration of **68** at 20 mg/kg significantly inhibited PAF-induced thrombocytopenia in rats. Compound **69** did not prevent *in vitro* chemotaxis at 10 μM. These results indicated that both lignans were potent and selective antagonists of PAF (Castro-Faria-Neto et al., 1993, 1995). Bioactivity-directed fractionation of the diethyl ether extract of *Piper puberulum* stems, a Chinese folk medicine used for the treatment of asthma and arthritic conditions, identified puberulins A (**70**) and C (**71**) as PAF receptor antagonists. Compounds **70** and **71** inhibited specific PAF receptor binding on isolated rabbit plasma platelet membrane with IC_{50} values of 7.3 and 5.7 μM, respectively (Zhang et al., 1995). Several dibenzocyclooctadiene lignans with PAF antagonistic activity were isolated from *Schisandra chinensis*, and were suggested to be the active principles of the crude drug in PAF-related inflammatory disorders, such as asthma, allergy, atopic dermatitis, and other inflammatory diseases (Jung et al., 1997; Lee et al., 1999).

Lignans with other biological activities

Other major biological activities related to lignans include immunosuppressive, anti-inflammatory, an-



tioxidative, and cardiovascular effects (Ríos et al., 2002). For example, podophyllotoxin as well as its derivatives were demonstrated to be potent immunosuppressive agents using the mouse allogeneic mixed lymphocyte reaction (MLR) method, with **72** being the most active (Gordaliza et al., 2000a). The neolignan magnoshinin (**73**) inhibited granuloma tissue formation in the adjuvant-induced inflammation mouse model and, after oral administration, showed a similar effect to that obtained with hydrocortisone at 60 mg/kg/day (Kimura et al., 1985). Antioxidative lignans have been covered by Potterat (1997) and Ríos et al. (2002). Selected examples include sesamolinol (**74**) and schisanhenol (**75**). Graminone B (**76**) from *Imperata cyclindrica* (Gramineae) inhibited the KCl-induced contraction of rabbit aorta without affecting NA-induced contraction, but the mechanism of its cardiovascular effects was unclear (Matsunaga et al., 1994). Other naturally occurring lignans with cardiovascular activity were reviewed recently by Ghisalberti (1997).

Conclusions and perspectives

As a group of diverse and widespread natural products, lignans have attracted much research interest due to their great variety of pharmacologic properties. Although few lignans are potent and safe enough to be used as therapeutical agents, these novel bioactive compounds provide new leads for drug development and broaden the scope of current investigations. To

date, only podophyllotoxin and some of its analogs (e.g. etoposide, teniposide and etopophos) have shown clear efficacy and safety and are used in the clinic as antiviral and anticancer drugs, respectively. Antitumor activity will undoubtedly continue to be the most clinically relevant property of lignans. Further rational drug design-based synthesis of podophyllotoxin and dihydrobenzofuran analogs is likely to produce therapeutic candidates for cancer chemotherapy. Among the other cited properties, hepatoprotective and PAF antagonistic activities may be of clinical relevance, as many of the bioactive lignans identified through bioactivity-directed fractionationand isolation originate from folk medicines used in treating related diseases.

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