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Lignans and neolignans as lead compounds

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Abstract

Many lignans and neolignans have served as lead compounds for the development of new drugs. Perhaps the best known example is podophyllotoxin, an antimitotic compound that binds to tubulin. Etoposide and teniposide are derived from podophyllotoxin, but their antitumoural activity is due to inhibition of topoisomerase II. Combination of both pharmacophores has led to compounds with a dual mechanism of action, such as azatoxin. Dihydrobenzofuran neolignans, based on the natural lead 3′,4-di-*O*-methylcedrusin, have also been investigated as potential antitumoural agents; the dimerisation product of caffeic acid methyl ester was the most active compound. Here too, he cytotoxic activity was due to inhibition of tubulin polymerisation. In addition, the same compounds showed antiangiogenic activity. Podophyllotoxin, as well as other types of lignans, such as dibenzylbutyrolactones related to arctigenin, dibenzocyclooctadiene-type lignans, and dibenzylbutanes, have been explored as leads for antiviral agents (also including HIV). Synthetic 8.*O*.4′-neolignans have been evaluated for their antileishmanial and antifungal properties. Detailed study of the antifungal properties of the phenylpropanoid moieties has resulted in the design of highly active arylpropanoid derivatives. Other examples where lignans have been used as lead compounds include enzyme inhibitors of phosphodiesterase IV and V, and 5-lipoxygenase, and for the development of hypolipidemic and antirheumatic agents.

Introduction

From a chemical point of view lignans and neolignans show an enormous structural diversity, although their molecular backbone consists only of two phenylpropane (C_6C_3) units. Nature itself offers a huge library of compounds, which could hardly be surpassed by modern combinatorial chemistry techniques. Many types of biological activity have been reported for members of this group of natural products. In many cases lignans and neolignans have served as a lead compound for the organic synthesis of derivatives to optimise the activity, and to study structure-activity relationships. The aim of this review is not to give a complete listing of all biological activities reported for lignans and neolignans, but to highlight some examples where a naturally occurring lignan or neolignan has been used as a template for the development of a potential new therapeutic agent. The discussion extends to lead compounds with cytotoxic, antiangiogenic, antiviral (including HIV), antileishmanial, antifungal, hypolipidemic and antirheumatic activity, as well as selective inhibitors of phosphodiesterases IV and V, and 5-lipoxygenase, covering literature between 1992 and 2002.

Podophyllotoxin as a lead for antitumoural agents

The first and perhaps the best known example of the use of a lignan as a lead compound is podophyllotoxin (**1**), a cytotoxic aryltetralin lactone originally obtained from *Podophyllum peltatum* L. and related species (Canel et al., 2000) (Figure 1). Podophyllotoxin interrupts the cell cycle by inhibition of microtubule assembly. The antimitotic activity of podophyllotoxin

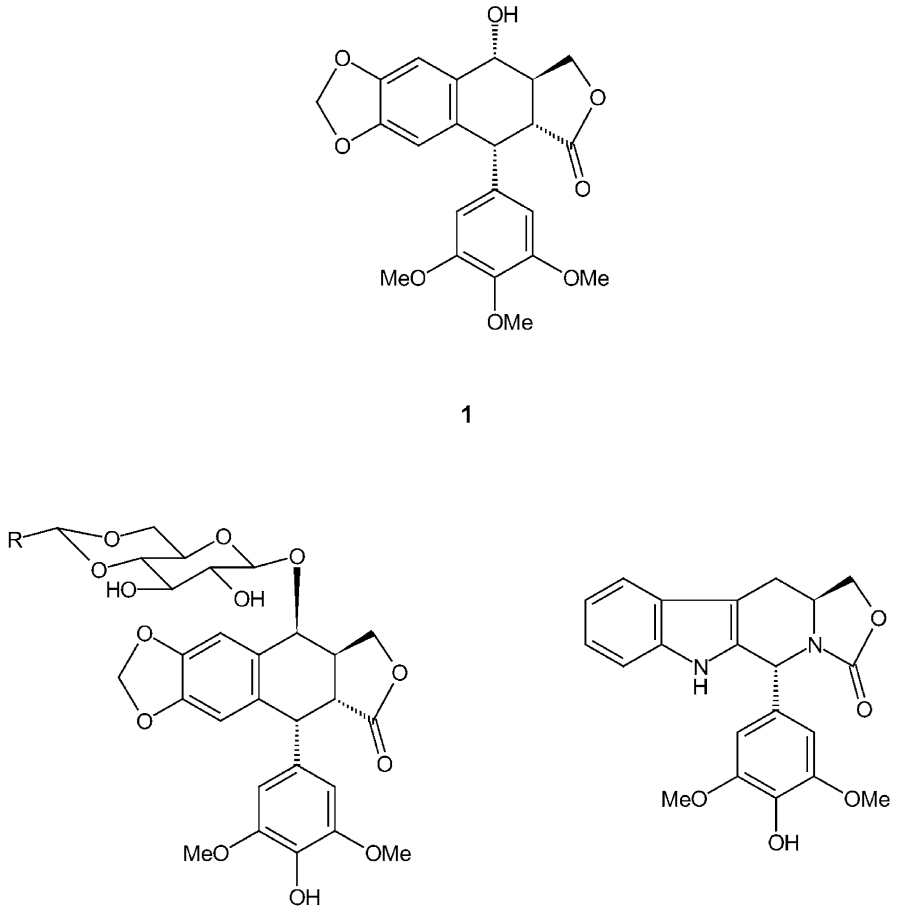


Figure 1. Podophyllotoxin (**1**), etoposide (**2**), teniposide (**3**) and azatoxin (**4**)

was reported at least half a century ago, and is due to reversible binding to tubulin. Podophyllotoxin was found to interact with tubulin at the colchicine binding site (Hamel, 1996). Compounds that inhibit tubulin polymerisation, such as the alkaloids vinblastine or vincristine, are commonly used as chemotherapeutic agents against cancer, and podophyllotoxin has also been explored as a lead compound for new antitumoural agents. Surprisingly, in addition to inhibitors of tubulin polymerisation, compounds with a completely different mechanism of action have also been obtained. Podophyllotoxin derivatives can be divided into two groups: (1) inhibitors of tubulin polymerisation, such as podophyllotoxin itself; and (2) inhibitors of DNA topoisomerase II, such as etoposide (**2**) and teniposide (**3**). DNA topoisomerases are involved in conformational and topological changes in DNA during replication. The main structural difference between these two groups is the presence of a small equatorial substituent in position 4 (C-ring) for inhibitors of tubulin polymerisation, and of a bulky substituent (such as a glucose moiety) in axial position for topoisomerase II inhibitors (Ter Haar et al., 1996). The latter group of compounds are 4β-congeners of podophyllotoxin, and are called the epipodophyllotoxins. Podophyllotoxin derivatives as potential new antitumoural agents have been the subject of recent reviews, which will not be repeated here (Shi et al.,

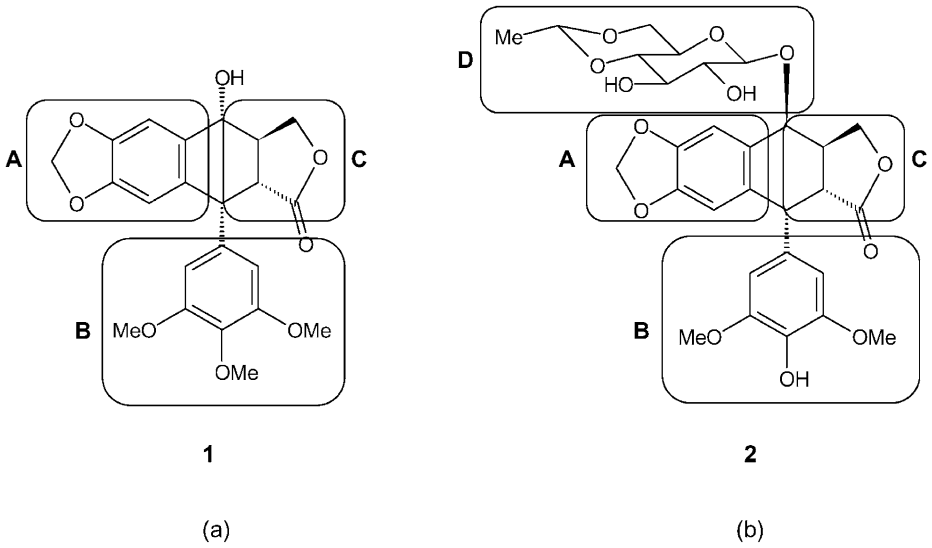


Figure 2. Pharmacophore for:
(a) inhibitors of tubulin polymerisation, binding at the colchicine site, e.g. podophyllotoxin (**1**): A, C = variable aromatic domains; C = linker
(b) topoisomerase II inhibitors, e.g. etoposide (**2**): A = planar (poly)aromatic array; B = pendant group; C = acyl functionality domain; D = variable substituent domain

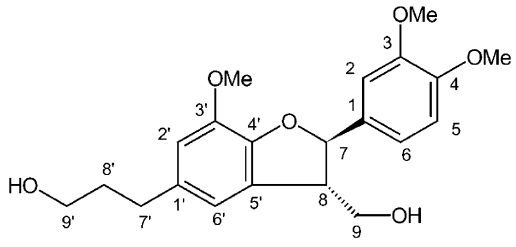
1998; Lee, 1999; Ramos et al., 1999; Gordaliza et al., 2000; Botta et al., 2001; Xiao et al., 2002). Podophyllotoxin analogues are still being explored nowadays by different research groups (e.g. Capilla et al., 2001; Gordaliza et al., 2001; Madrigal et al., 2002; Roulland et al., 2002). It has been observed that treatment of cancer patients with cytotoxic agents also diminished their immune responses. Therefore cytotoxic agents such as podophyllotoxin derivatives (cyclolignans) have been investigated as immunosuppressive drugs to prevent rejection of transplanted organs (Gordaliza et al., 1996, 1997).

The pharmacophore for inhibitors of tubulin polymerisation, binding at the colchicine site, includes two variable aromatic domains kept in a non-planar arrangement by different linkers (Figure 2a) (Ter Haar et al., 1996; Miller et al., 1998). Careful analysis of structurally diverse topoisomerase II inhibitors (not restricted to podophyllotoxin derivatives), on the other hand, has resulted in the construction of a composite pharmacophore with four domains: a planar (poly)aromatic array responsible for interaction with DNA; a pendant group for minor groove binding; a variable substituent domain, also for minor groove binding; and the acyl functionality domain dictating the conformational relationship between the three other domains (Figure 2b) (Madalengoitia et al., 1997).

Interestingly, molecular hybridisation of etoposide (**2**) and the alkaloid ellipticine, an inhibitor of topoisomarase II, has resulted in the synthesis of azatoxin (**4**), a highly potent cytotoxic compound with a dual mechanism of action, i.e. inhibition of tubulin polymerisation and topoisomerase II (Leteurtre et al., 1992; Solary et al., 1993). Azatoxin can be considered as a new lead for antitumoural agents, which is still currently being explored. Structure – activity relationships established for azatoxin have shown a much closer similarity to podophyllotoxin than to ellipticine (Ramos et al., 1999).

Dihydrobenzofuran neolignans as a lead for antitumoural agents

In addition to podophyllotoxin, other types of lignans and neolignans have also been characterised as inhibitors of tubulin polymerisation. In our laboratory we have investigated a series of dihydrobenzofuran neolignans as potential antitumoural agents, based on the natural lead 3′,4-di-*O*-methylcedrusin (**5**, biogenetic numbering) (Figure 3). This compound was identified as one of the minor constituents of the red latex, called ‘dragon’s blood’ (‘sangre de drago’) in traditional medicine in South America, which is obtained by slashing the bark of various *Croton* species (Euphorbiaceae). 3′,4-Di-*O*-methylcedrusin (**5**) is a



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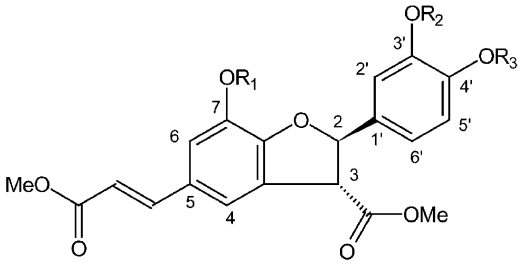

6 R_1, R_2, R_3 = H

7 R_1, R_2 = Me, R_3 = H

8 R_1, R_2, R_3 = Me

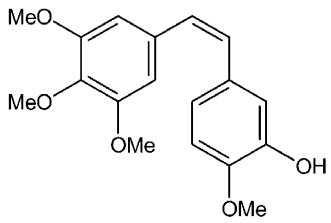

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Figure 3. Dihydrobenzofuran neolignans **6–8** derived from 3′,4-di-*O*-methylcedrusin **5**, and combretastatin A-4 (**9**)

dihydrobenzofuran derivative, a relatively rare class of neolignans (in neolignans the two C_6C_3 units are not linked by a β-β bond, in contrast to the lignans). This compound was found to act as an inhibitor of cell proliferation (Pieters et al., 1993). In order to explore the potential antiproliferative and antitumoural activity of 3′,4-di-*O*-methylcedrusin (**5**), obtained in low yield from its natural source, a synthesis was devised from various C_6C_3 precursors, leading to both **5** and a series of synthetic dihydrobenzofuran neolignans. Their cytotoxicity was determined in an *in vitro* human disease-oriented tumour cell line screening panel. A structure-activity relationship could be established, and the mechanism of action was determined (Pieters et al., 1999).

All dihydrobenzofuran neolignans synthesized were evaluated in the *in vitro* human disease-oriented tumour cell line screening panel developed at the NCI, allowing determination of the average log GI_{50} values (GI_{50} being the molar drug concentration required for half growth inhibition) calculated from all cell lines tested, as well as the log GI_{50} values for a series of representative cell lines. The complete set of data obtained in this screening panel showed that the leukemia cell lines and the breast cancer cell lines were relatively more sensitive to the cytotoxic dihydrobenzofuran neolignans than were other cell lines. Three dihydrobenzofuran lignans [**6**, **7** and **8**(2*R*,3*R*)] showed an average log $GI_{50} < -5$ (corresponding to average GI_{50} values of 0.3, 3.3 and 5.1 μM, respectively) and were selected for further evaluation as potential antitumoural agents (Figure 3, IUPAC numbering for benzofurans). Compound **6** was the dimerisation product of caffeic acid methyl ester, containing a 3′, 4′-dihydroxyphenyl moiety and a hydroxyl group in position 7 of the dihydrobenzofuran ring. Compound **7** was the dimerisation product of ferulic acid methyl ester, with only one free hydroxyl group left in position 4′; compound **8** was the methylated derivative of **7**. All three compounds still contained the methyl ester functionality and the double bond in the side chain. Apparently, methylation of the hydroxyl groups reduced activity, since the caffeic acid derivative **6** was 10 times more potent than the ferulic acid derivative **7**. Methylation of **7** led to a further loss of activity. Of all dihydrobenzofuran neolignans tested, compound **6** showed the greatest cytotoxicity against every cancer cell line examined. Against three breast cancer cell lines (MDA-MB-435, MDA-N and BT-549), the GI_{50} for **6** was < 10 nM. Submicromolar GI_{50} values were observed against additional leukemia and breast cancer cell lines evaluated in the NCI screen as follows: 0.033 μM (CCRF-CEM), 0.081 μM (MOLT-4), 0.055 μM (RPMI-8226), 0.069 μM (SR) (leukemia cell lines); 0.029 μM (MCF7), 0.054 μM (MCF7 / ADR-RES), 0.050 μM (MDA-MB-231 / ATCC),

0.045 μM (HS 578 T) (breast cancer cell lines). Reduction of the double bond in the C_3 side chain caused a greater than 10-fold decrease of activity, while additional reduction of the methyl ester functionality to a primary alcohol led to an additional 10-fold loss in activity. The cytotoxic activity was found to be related to the stereochemistry of the dihydrofuran ring, the 2*R*,3*R*-isomer being the more active one. The corresponding benzofuran derivatives were inactive.

For all compounds tested, including the most active ones, no TGI (total growth inhibition) or LC_{50} (50% cell kill) level was reached for most cell lines (log TGI and log $LC_{50} > -4.00$, 100 μM being the highest concentration tested). This indicated that their mechanism of action was cytostatic rather than cytotoxic within the 48 h time frame of the assay. The COMPARE algorithm (Paull et al., 1989), a program that compares a complete set of cell sensitivities to those of standard agents or other agents present in the NCI database, showed a correlation (correlation coefficient = 0.735) between **6** and a number of combretastatins (see also below), which are known to act as antimitotic compounds by inhibiting tubulin polymerisation (Hamel, 1996; Ter Haar et al., 1996). Correlation coefficients > 0.6 can be considered significant. In addition, the flat configuration of dose-response curves, obtained when TGI or LC_{50} values are not reached in the screening assays, is observed with many antitubulin agents.

When examining the effect of the test compounds on the polymerisation of purified bovine brain tubulin, compound **6** inhibited the extent of polymerisation by 50% at a concentration of 13 ± 1 (SD) μM (n = 3), while the IC_{50} value for combretastatin A-4 (**9**) (Figure 3), used as a positive control, was 1.2 ± 0.03 (SD) μM. Chromatographic resolution of **6** into its enantiomers allowed their evaluation for antitubulin activity, too. The 2*R*,3*R*-enantiomer yielded an IC_{50} value of 6.0 ± 0.4 μM, while the 2*S*,3*S*-enantiomer was essentially inactive ($IC_{50} > 40$ μM). Combretastatin A-4 as well as compound **6** inhibited the binding of [^{3}H]colchicine to tubulin, and their relative activities were quantitatively consistent with their relative effects on the polymerisation reaction. In reaction mixtures containing 1.0 μM tubulin and 5.0 μM [^{3}H]colchicine, binding of radiolabeled drug to the protein was inhibited 88 ± 4% by 1.0 μM combretastatin A-4, and total inhibition occurred with 5.0 μM combretastatin A-4. Combretastatin A-4 is one of the most potent competitive inhibitors of colchicine binding to tubulin known to date. Compound **6** was less potent: 14.0 ± 0.2% inhibition occurred with the compound at 5.0 μM, and 53 ± 3% at 50 μM **6**.

Compound **6** was also evaluated for its effects on the growth of HL 60 human leukemia cells. An IC_{50} value of 0.2 μM for **6** at 24 h on the growth of this cell line (increase in cell number from time zero) was obtained. Next the effect on the mitotic index at 10 μM was examined. After 24 h of growth, 35% of the cells displayed a mitotic configuration (condensed chromosomes), as compared with 2% in the control culture. The enantiomers were also examined for inhibitory effects on the growth of the HL 60 cells. The same relative activities were observed as with inhibition of tubulin polymerisation. The IC_{50} value obtained with the 2*R*,3*R*-enantiomer was 0.08 μM, versus 0.6 μM with the 2*S*,3*S*-enantiomer. These values were obtained with freshly dissolved compounds. At least one of the chiral centres of the enantiomers probably racemize in solution, although other mechanisms of degradation, such as aromatisation or ring opening of the dihydrobenzofuran ring, may not be excluded. Preliminary experiments have yielded smaller differences when older solutions of the enantiomers were compared than when freshly prepared solutions were evaluated. Finally, the 2*R*,3*R*-enantiomer was similar to racemic **6** in causing a marked increase in the mitotic index of HL 60 cells. Compounds **6**, **7** and **8**(2*R*,3*R*) were also evaluated in the hollow fiber assay developed at the NCI. This is a preliminary *in vivo* screening tool for assessing the potential anticancer activity of compounds selected in the *in vitro* cell screen. However, none of the compounds tested was sufficiently active for further *in vivo* testing in standard subcutaneous xenograft models. Because this inactivity *in vivo* might be related to enzymatic hydrolysis of the methyl esters, more bulky esters such as the *n*-propyl, *n*-butyl and *t*-butyl derivatives were also prepared, but this caused over a 10-fold decrease in *in vitro* cytotoxicity. The loss of activity *in vivo* might also be related to instability and ring opening of the dihydrobenzofuran ring.

In conclusion, the dihydrobenzofuran neolignan **6** appeared to inhibit mitosis at μM concentrations in cell culture through a relatively weak interaction at the colchicine binding site of tubulin, and these activities in the racemate probably derive from activity limited to the 2*R*,3*R*-enantiomer. These dihydrobenzofuran neolignans (2-phenyl-dihydrobenzofuran derivatives) constitute a new group of antimitotic agents that inhibit tubulin polymerisation, showing the same pharmacophore consisting of two variable aromatic

domains kept in a non-planar arrangement by a linker, as discussed above for podophyllotoxin.

Dihydrobenzofuran neolignans as a lead for antiangiogenic agents

Angiogenesis or neovascularisation is a complex process involving the activation, adhesion, proliferation and transmigration of endothelial cells from pre-existing blood vessels. It plays a role in normal physiological processes such as wound healing, but also in a number of pathological processes, for instance diabetic retinopathy, arthritis, and the growth of solid tumours. Angiogenesis is therefore considered as a potential target for antitumoral therapy. Characterisation of new antiangiogenic leads may yield new therapeutic agents in this area. The antiangiogenic activity of some synthetic dihydrobenzofuran neolignans was evaluated in the CAM (chorioallantoic membrane) assay, an *in vivo* model for angiogenesis. This assay is based upon the formation of a chorioallantoic membrane, in which neovascularisation takes place, in fertilized chicken's eggs at a certain stage of the development of the embryo. Agarose pellets impregnated with the test compound are placed onto the vascular membrane of opened eggs, and the influence on angiogenesis is evaluated by observing the avascular zone surrounding the pellet. Antiangiogenic activity is expressed as a score where 0 = no or weak effect, 1 = medium effect and 2 = strong effect (the capillary-free zone is at least twice as large as the pellet). Membrane irritation and embryotoxicity can also be evaluated (Apers et al., 2002).

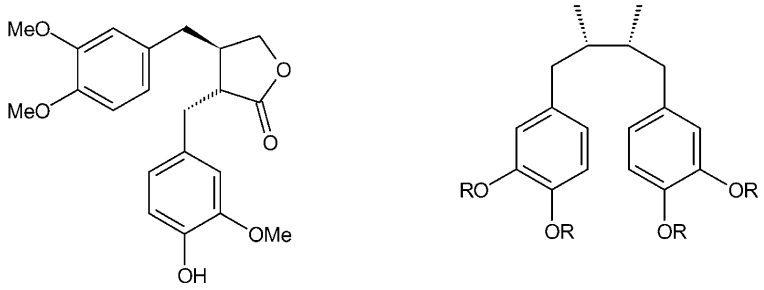
Dihydrobenzofuran neolignan **6** showed an antiangiogenic score of more than 1 when tested at a dose of 20 μg/pellet (or 50 nmol/pellet). When evaluating both enantiomers separately, it turned out that the (2*R*,3*R*)-isomer exhibited an especially strong antiangiogenic activity, with a score of 1.5 ± 0.1. However, also a membrane irritating effect (about 50%) was also observed at 20 μg/pellet. Compound **6**(2*R*,3*R*) still showed antiangiogenic properties (score 0.8) at 5 μg/pellet. Methylation and reduction of the parent compound reduced the antiangiogenic activity, as observed previously for the antimitotic activity and the inhibition of tubulin polymerisation (see previous section). Other antimitotic agents and inhibitors of tubulin polymerisation, such as colchicine or vincristine, also inhibited angiogenesis in the CAM-assay (Paper, 1998). Since the proliferation of endothelial cells is involved in angiogenesis, the antiangiogenic effect of antimitotic agents may be related to their antiproliferative action on endothelial cells. Dihydrobenzofuran **6** can be considered as a new lead for antiangiogenic agents, which deserves further exploration.

Lignans as antiviral lead compounds

In addition to its cytotoxic activity, podophyllotoxin is also known for its antiviral properties (Canel et al., 2000). The antiviral activity (including HIV) of naturally occurring lignans and synthetic analogues was reviewed recently by Charlton (1998), covering the literature up to 1997, and this work will not be repeated here. He concluded that there were several modes of antiviral activity associated with lignans: tubulin binding (inhibition of tubulin polymerisation interferes with the formation of the cellular cytoskeleton and with some critical viral processes), reverse transcriptase inhibition, integrase inhibition, and topoisomerase inhibition (although the latter association appeared to be less strong). Whereas podophyllotoxin and its derivatives were the most prominent representatives of the tubulin binding lignans, inhibition of reverse transcriptase was observed for various classes of lignans, such as dibenzylbutyrolactones, dibenzylbutanes, dibenzocyclooctadienes and aryltetralins. Dibenzylbutyrolactones derived from arctigenin (**10**) were active as inhibitors of viral integrase (Figure 4).

More recently, the plant lignan nordihydroguaiaretic acid (**11**) was found to suppress HIV-1 replication in infected cells by preventing proviral transcription and HIV Tat-transactivated transcription. A series of methylated derivatives were prepared, and it was found that tetramethyl nordihydroguaiaretic acid (**12**) was more active than the original lead (Hwu et al., 1998). The same derivatives, as well as tetraacetyl nordihydroguaiaretic acid, were found to be potentially useful in the treatment of papillomavirus infections and their associated induced human cancers (Craigo et al., 2000). In addition, it was shown that tetramethyl nordihydroguaiaretic acid inhibited melanoma *in vivo* (Lambert et al., 2001).

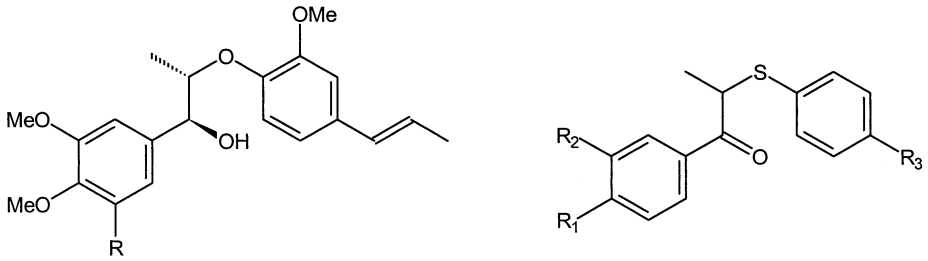
An extensive study of the antiviral activity of a series of 1-arylnaphthalene and 1-aryl-1,2-dihydronaphthalene lignans and their analogues was carried out by Cow et al. (2000). The compounds were tested against the human cytomegalovirus, but the antiviral activity did not extend into the nanomolar range and was often paired with high cytotoxicity.



10 **11** R = H

12 R = Me

Figure 4. Arctigenin (**10**), nordihydroguaiaretic acid (**11**) and its tetramethyl derivative (**12**)



13 R = OMe **15** R_1, R_2 = H R_3 = Cl

14 R = H **16** R_1, R_2 = OMe R_3 = Me

17 R_1, R_2 = OMe R_3 = Cl

Figure 5. Surinamensin (**13**), virolin (**14**) and antileishmanial β-ketosulfides **15** – **17**

O.4′-Neolignans as a lead for antileishmanial agents

The 8.*O*.4′-neolignans are a relatively small group of neoligans that are only found in the Myristicaceae family. A series of synthetic 8.*O*.4′-neolignans was synthesized, based on the active principles isolated from *Virola surinamensis*, surinamensin (**13**) and virolin (**14**) (Figure 5), responsible for blocking of penetration of cercariae of *Schistosoma mansoni* in mice. Compounds with ether linkages and their corresponding C-8 sulphur and nitrogen analogues were obtained and evaluated for their activity against *Leishmania donovani*, one of the causative agents of leishmaniasis (Barata et al., 2000). The highest selective activity against amastigotes was found in those compounds with sulphur bridges, i.e. the β-ketosulfides 2-(4-chlorophenoxy)-propiophenone (**15**), (3,4-dimethoxy)-8-(4′-methylthiophenoxy)-propiophenone (**16**), and (3,4-dimethoxy)-8-(4′-chlorothiophenoxy)-propiophenone (**17**), suggesting that the C-8 sulphur bond may play a role in their antileishmanial activity. Only compound **16** was significantly active *in vivo*, producing 42% inhibition of *Leishmania donovani* amastigotes in the liver of infected mice, at 100 mg/kg orally once a day during five consecutive days. A series of twenty compounds, both active and inactive, were analysed using the molecular mechanics method (MM2) in order to determine the most probable active conformers that may fit the putative receptor (Costa et al., 1997). Because of the critical role of the enzyme adenosine kinase, one of the key enzymes of the purine-salvage pathway in the metabolism of parasitic protozoa, including *Leishmania donovani* (which are not capable of *de novo* purine biosynthesis), it was assumed that the active neolignans interacted with the arginine residue, present at the adenosine binding site of leish-

manial adenosine kinase, and inactivated the enzyme. The most probable conformations for this interaction were discussed (Costa et al., 1999). More recently the same authors used pattern recognition techniques to establish structure-activity relationships between calculated molecular properties of the same series of compounds and activity against *Leishmania donovani*. Some of these neolignans were fitted with the arginine residue in the adenosine kinase enzyme in order to define their binding site (Costa et al., 2001).

However, these studies are based on the hypothesis that adenosine kinase is the target enzyme for these antileishmanial neolignans. It is interesting to note that leishmanicidal activity has also been reported for a series of combretastatin analogues (Del Rey et al., 1999); combretastatins show some structural similarity with 8.*O*.4′-neolignans. Although the mechanism of their action against *Leishmania* species has not been reported, combretastatins are known as potent inhibitors of tubulin polymerisation (see above). Microtubule inhibitors have been considered to be promising lead-drug candidates for parasitic diseases such as leishmaniasis (Callahan et al., 1996). The tubulins of humans and parasites have significant differences in their primary amino acid sequence and polymerisation properties, and molecules that bind to human tubulin, such as colchicin, do not necessarily bind to leishmanial tubulin. It has been observed that vinca-site agents such as vinblastine bind to leishmanial tubulin, in contrast to colchicine-site agents. Paclitaxel, a potent inhibitor of cell replication that is active in inhibiting tubulin depolymerisation into stable microtubules, and is currently in clinical use as an anticancer drug, is known to be active against protozoan parasites, including *Leishmania donovani* amastigotes (Werbovetz et al., 1999; Jayanarayan and Dey, 2002). It is an interesting speculation that inhibition of tubulin polymerisation might be involved in the antileishmanial activity of the 8.*O*.4′-neolignans and the combretastatin analogues mentioned above. Indeed, preliminary mode of action studies on the β-ketosolfides discussed above suggested microtubule inhibition, but further studies are needed to fully elucidate the mechanism of action (Barata et al., 2000).

8.O.4′-Neolignans as a lead for antifungal agents

A series of eighteen synthetic racemic 8.*O*.4′-neolignans with six different substitution patterns in the aromatic rings, and with a ketone or a hydroxyl functionality in the C – C – O chain linking the two rings, were evaluated for their antifungal activity (Zacchino et al., 1997). Only those compounds with a free alcohol functionality in the C – C – O linker exhibited a broad spectrum of activity, with (±)-erythro-3,4-(methylenedioxy)-7-hydroxy-1′-allyl-3′, 5′-dimethoxy-8.*O*.4′-neolignan (**18**) being the most active compound, against the dermatophytes *Microsporum canis* (MIC 20 μg/ml), *Microsporum gypseum* (MIC 50 μg/ml), *Tricophyton mentagrophytes* (MIC 100 μg/ml), *Tricophyton rubrum* (MIC >250 μg/ml), and *Epidermophyton floccosum* (MIC 5 μg/ml) (Figure 6). *Erythro*-compounds were up to three times more active than their *threo*-isomers. Substitution with two methoxyl groups instead of the methylenedioxy substituent in neolignan **18** reduced the activity eight times. No activity was observed against *Candida albicans*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, *Aspergillus niger*, *Aspergillus fumigatus*, or *Aspergillus flavus*. Both aromatic rings were necessary for the antifungal activity, since (±)-1-[3,4-(methylenedioxy)phenyl]-1-hydroxy-propane as well as 4-allyl-2,6-dimethoxyphenol, corresponding to both aromatic moieties of neolignan **18**, were inactive. *In vitro* studies on the mode of action strongly suggested that the antifungal activity of the 8.*O*.4′-neolignans was due to inhibition of cell wall polymer synthesis or assembly. Although the antifungally active compounds were able to inhibit the enzyme (1,3)-β-glucan synthase, an enzyme that catalyses the synthesis of the major cell wall polymer (1,3)-β-glucan, comparison with the results of the antifungal assays led to the conclusion that inhibition of this enzyme was not the mechanism of action. In addition, the antifungally inactive ketone derivatives also showed similar glucan synthase inhibition (Zacchino et al. 1998).

A more systematic study of the role that the phenylpropanoid moieties play in the antifungal activity of the dimeric neolignans confirmed that this activity could not be attributed to the monomeric units (Zacchino et al. 1999). Among the phenylpropanoids tested, only α-haloketoderivatives showed a pronounced antifungal activity against dermatophytes, but because of the possible vesicant and lachrymatory properties of these reactive compounds, they were not considered as new antifungal leads. However, when the phenyl ring in a monomeric phenylpropanoid derivative was replaced by a naphthyl or a phenanthryl ring, strong antifungal activity was also observed for structures that did not possess a halogen. In contrast

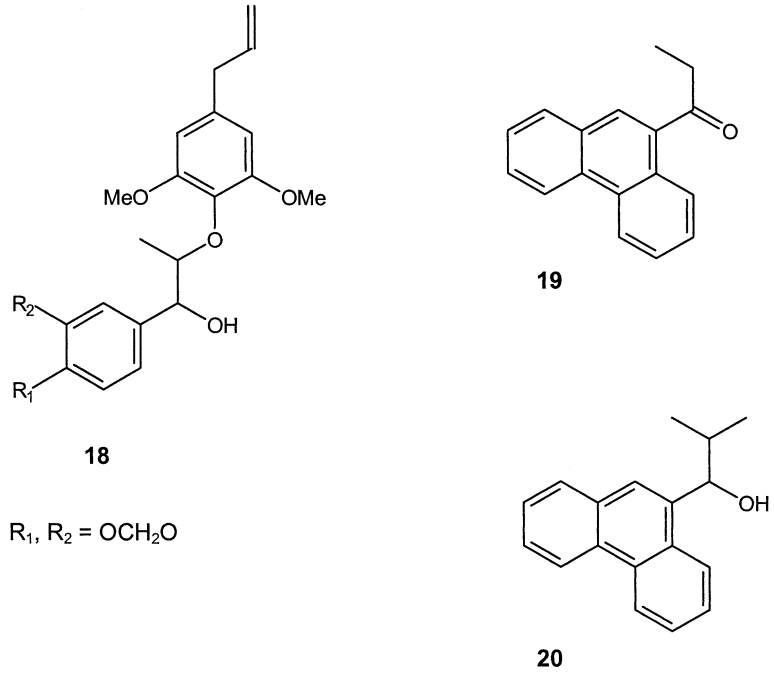


Figure 6. Antifungal 8.*O*.4′-neolignan **18** and phenylpropanoids **19** and **20**

to the 8.*O*.4′-neolignans, ketones as well as alcohols were active. Compounds such as **19** and **20** (Figure 6) displayed antifungal properties similar to amphotericin B or ketoconazole and were considered as leads for the development of new topical antifungal agents.

1-Arylnaphthalene lignans as leads for selective phosphodiesterase IV and V inhibitors

Cyclic nucleotide phosphodiesterases are the enzymes responsible for the hydrolysis of the cyclic nucleotides cAMP and cGMP to the respective 5′-mononucleotides. Phosphodiesterases have been classified in at least 11 identified families to date. Selective phosphodiesterase IV inhibitors, resulting in the increase in intracellular levels of cAMP, which contributes to both the relaxation of airway smooth muscle cells and the prevention of proinflammatory cell activation, are considered as potential antiasthmatic agents. Arylnaphthalene lignans were found to show phosphodiesterase IV inhibitory activity. In a series of synthetic 1-aryl-2,3-bis(hydroxymethyl)naphthalene lignans the most potent and selective compounds were those bearing an *N*-alkylpyridone ring at C-1, such as 6,7-diethoxy-2,3-bis(hydroxymethyl)-1-[1-(2-methoxyethyl)-2-oxo-pyrid-4-yl]naphthalene (**21**) (phosphodiesterase IV IC_{50} 0.057 μM, selectivity ratio phosphodiesterase III / IV: 1000) (Iwasaki et al., 1996) (Figure 7). The change of the phenyl ring to a pyridone ring was based upon the observation that the amide group on the C-1 phenyl ring in compounds such as **22** played an important role in the phosphodiesterase IV inhibitory activity, and markedly improved the selectivity ratio. 6,7-Dimethoxy instead of 6,7-diethoxy substitution in **21** resulted in a severe loss of phosphodiesterase IV inhibitory activity; the 6-methoxy, 7-ethoxy derivative, however, was also active. Replacement of the 2,3-bis(hydroxymethyl) groups with 2,3-bis(acetoxymethyl) groups resulted in an important loss of activity. When given intravenously to anesthetised guinea pigs the same compound showed a pronounced reduction of histamine-induced and antigen-induced bronchoconstriction (ED_{50} 0.08 and 2.3 mg/kg, respectively) . Minimal cardiovascular side effects (increase in heart rate), were observed due to inhibition of phosphodiesterase III, and compound **21** was selected for further evaluation as an antiasthmatic agent.

Based on this work, Ukita et al. (1999a) synthetised a series of 1-pyridylnaphthalene derivatives, containing a naphthalene part (rings A and B), a pyridyl group (ring C), and a heterocyclic compound with a carbonyl group (ring D) (**23**) (Figure 7).

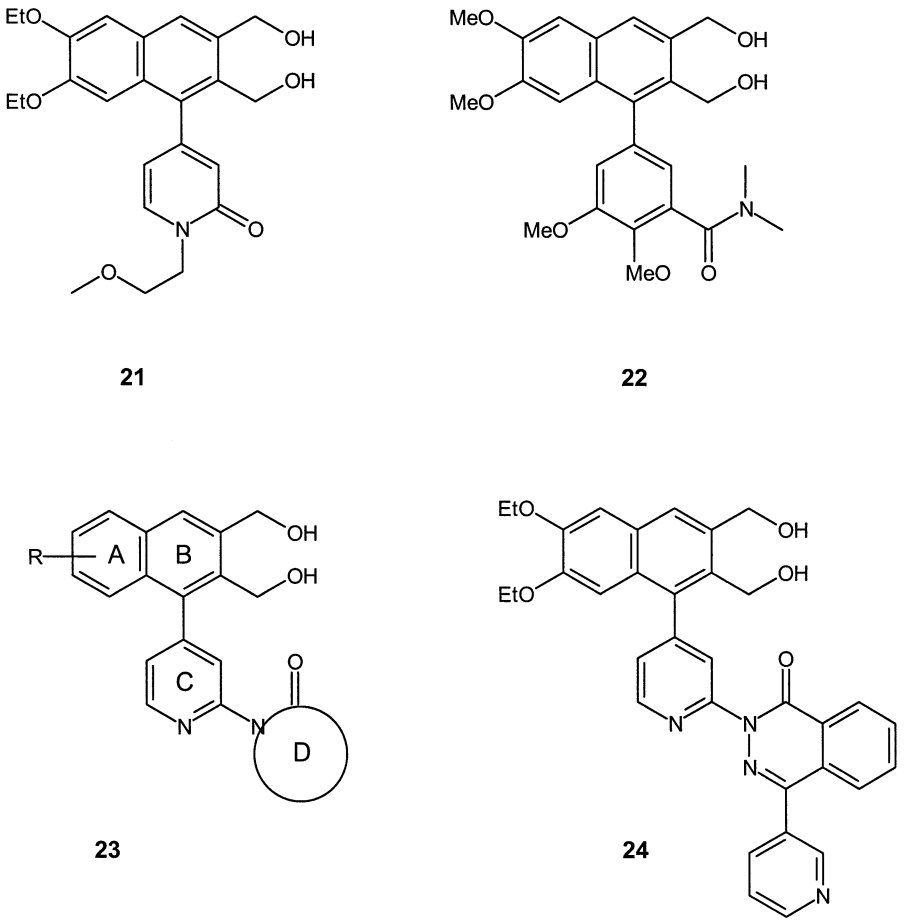


Figure 7. 1-Arylnaphthalene lignans as leads for phosphodiesterase IV inhibitors (**21** – **24**)

Among the compounds tested, 2,3-bis(hdroxymethyl)-6,7-diethoxy-1-{2-[1(2H)-phthalazinon-4-(3-pyridyl)-2-yl]-4-pyridyl}naphthalene (**24**) was the most promising compound (phosphodiesterase IV IC_{50} 0.13 nM, selectivity ratio phosphodiesterase III / IV: 14000). Compound **24** showed a pronounced reduction of histamine-induced and antigen-induced bronchoconstriction in the guinea pig model without significant changes in heart rate (ED_{50} 0.033 and 0.063 mg/kg, respectively), and was selected for further evaluation as an antiasthmatic agent.

Selective inhibitors of phosphodiesterase V, a cGMP-specific phosphodiesterase, have therapeutic potential for the treatment of hypertension, angina, congestive heart failure, and erectile dysfunction, because of the vasorelaxant activity of cGMP. Both selective phosphodiesterase IV and V inhibitors derived from the common scaffold 1-arylnaphthalene lignan have been reported, the spectra of phosphodiesterase inhibition depending entirely on the chemical modification of the substituents. In a series of synthetic 1-arylnaphthalene lignans, the most potent and selective phosphodiesterase V inhibitor was 1-(3-bromo-4,5-dimethoxyhenyl)-5-chloro-3-[4-(2-hydroxymethyl)-1-piperazinylcarbonyl]-2-(methoxycarbonyl) naphthalene (**25**) (IC_{50} 6.2 nM, selectivity ratio (phosphodiesterases I, II, III and IV: >16000) (Figure 8). Potent effects on the relaxation of rat aortic rings precontracted with phenylephrine were observed (EC_{50} 0.10 μM) (Ukita et al.1999b).

Superimposition of cyclic GMP (**26**) and 1-arylnaphthalenes such as **25** showed that the naphthalene ring in **25** overlapped the purine ring of cGMP (**26**), and that the pendant phenyl ring at the 1-position in **25** may fill the space occupied by the cyclic phosphate group (Figure 8). In addition, the importance of the amide carbonyl group at the 3-position of the naphthalene ring in **25** might be related to its structural sim-

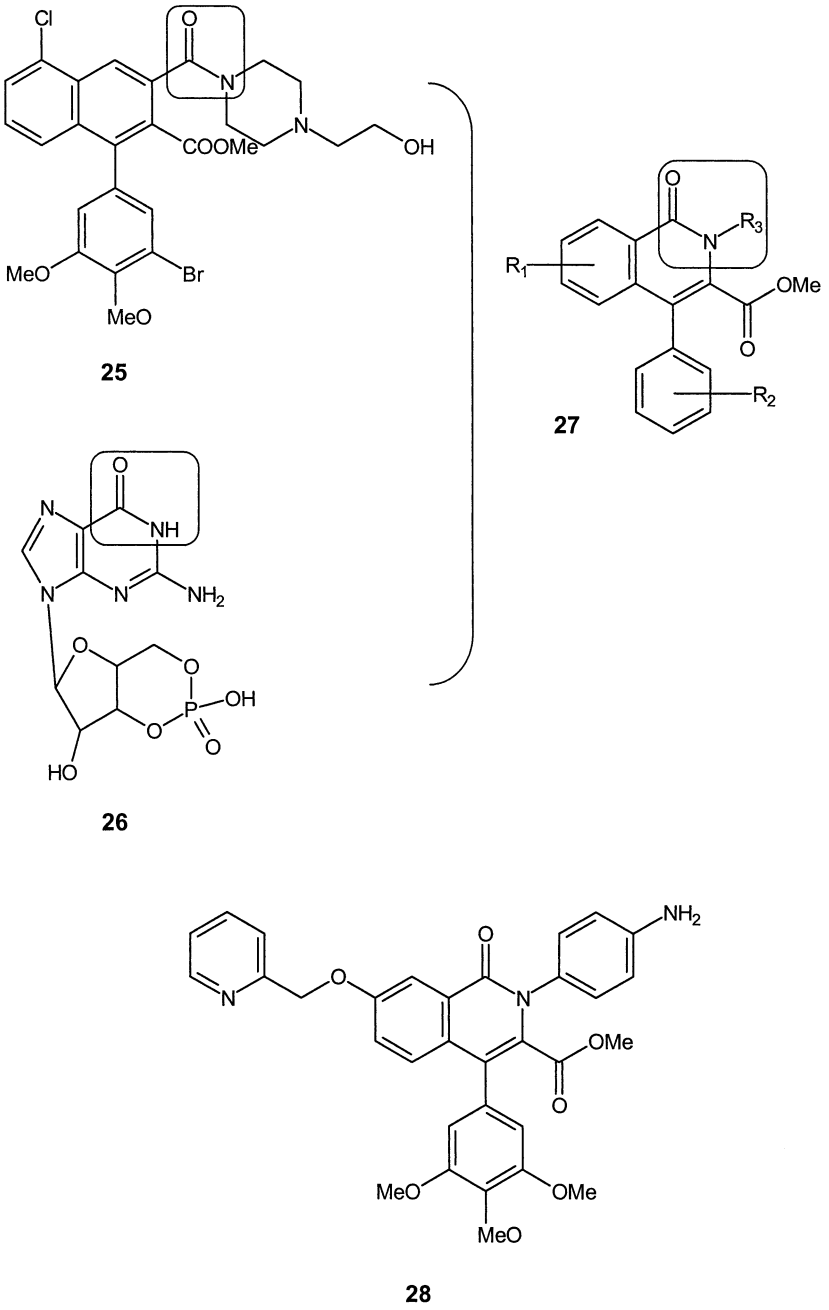


Figure 8. 1-Arylnaphthalene lignans as leads for phosphodiesterase V inhibitors (**25** – **28**)

ilarity with the amide-like functionality present in the guanine ring. A series of 4-aryl-1(2H)-isoquinolinone derivatives (**27**) was therefore designed, in which the amide carbonyl group at C-3 of the naphthalene ring was moved into the bicylic ring system (Ukita et al., 2001). The 1(2H)-isoquinolone derivatives were disclosed as a new structural class of potent and selective phosphodiesterase V inhibitors. Of all compounds tested, **28** showed the most potent and selective activity (IC_{50} 1.0 nM, selectivity ratio phosphodiesterase I / V: 1300; II / V: > 10000; III / V: > 10000; IV / V: 4700; VI / V: 28). Relaxant effects were observed on isolated rabbit corpus cavernosum (EC_{30} 7.9 nM), and compound **28** was selected for further biological and

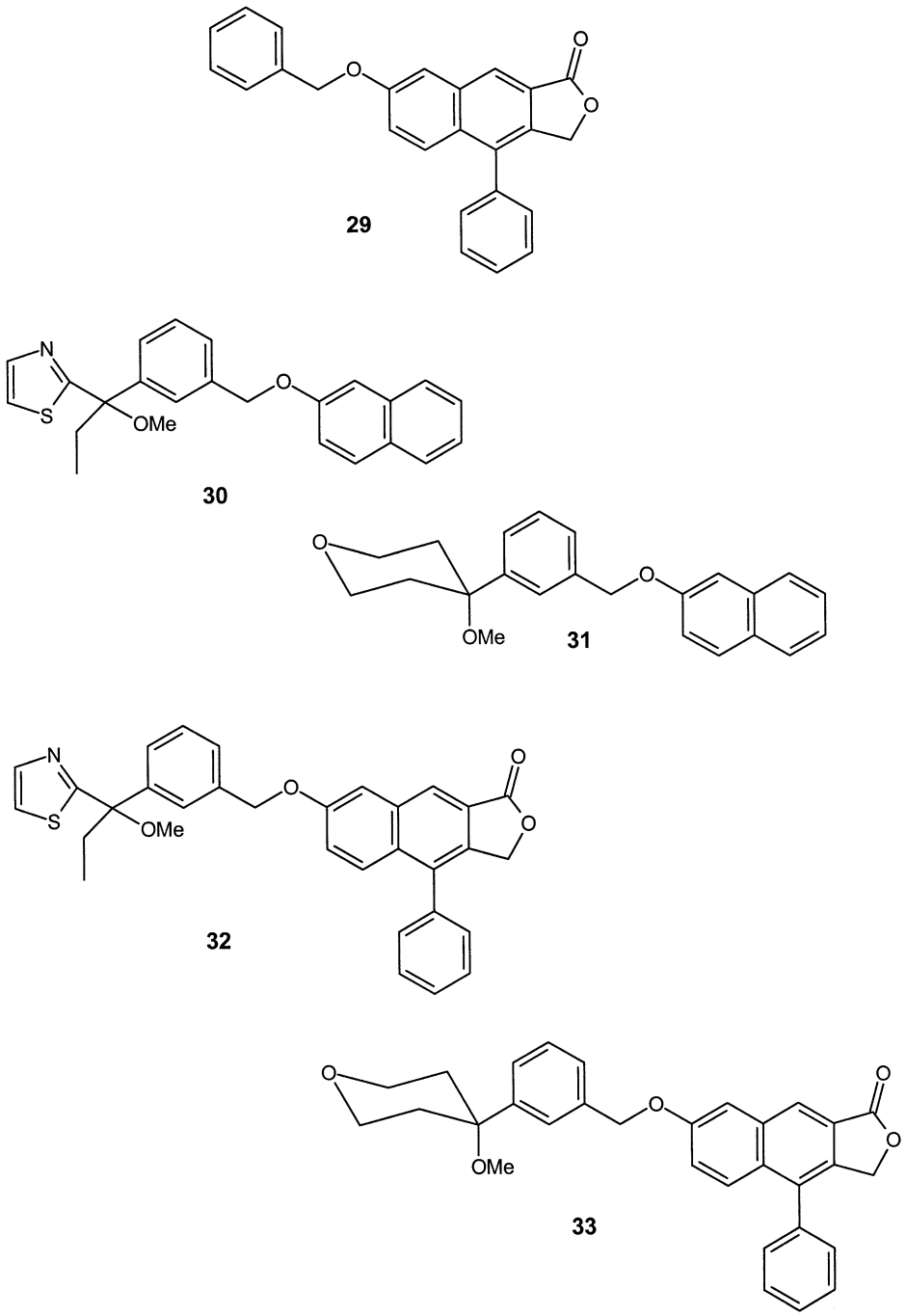
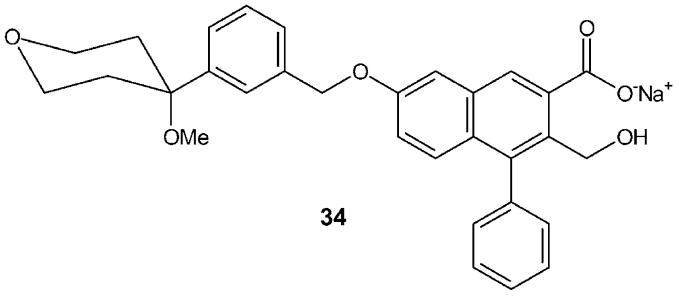


Figure 9. Naphthalenic lignan lactones as a lead for nonredox 5-lipoxygenase inhibitors (**29** – **33**)



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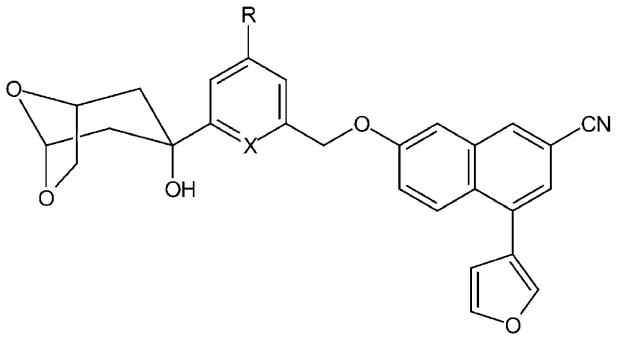


35 R = H, X = CH

36 R = H, X = N

37 R = F, X = CH

Figure 10. Naphthalenic lignan lactones as a lead for nonredox 5-lipoxygenase inhibitors (**34** – **37**)

pharmacological evaluation in view of the treatment of erectile dysfunction.

Naphthalenic lignan lactones as a lead for nonredox 5-lipoxygenase inhibitors

Members of the class of naphthalenic lignan lactones, exemplified by structure **29**, were identified as the result of a wide screening effort as moderately potent, nonredox inhibitors of the enzyme 5-lipoxygenase (Figure 9). This enzyme is involved in the biosynthesis of leukotrienes from arachidonic acid, and selective 5-lipoxygenase inhibitors may be useful in the treatment of asthma, inflammatory diseases and rheumatoid arthritis. Independently, naphthalene derivatives containing an additional phenyl ring and a thiazol (e.g., compound **30**) or a tetrahydropyran moiety (e.g. compound **31**) were also reported as new classes of 5-lipoxygenase inhibitors. Combination of structural elements of both classes of compounds and the naphthalenic lignan lactone led to the synthesis of **32** and **33** (Ducharme et al., 1994). Both compounds retained their potencies with either an oxymethylene (structures not shown) or a methyleneoxy linker. Compound **33**, being more active than **32**, inhibited the oxidation of arachidonic acid by by recombinant human 5-lipoxygenase (IC_{50} 14 nM), and the production of leukotriene B4 in human polymorphonuclear leukocytes (IC_{50} 1.5 nM) and in human whole blood (IC_{50} 50 nM). No significant inhibition of human 15-lipoxygenase or porcine 12-lipoxygenase, nor binding to human 5-lipoxygenase-activating protein was observed. Compound **33** was found not to be active *in vivo* in a rat pleurisy model because of its poor absorption. However, the open hydroxy acid form **34** was well absorbed, and showed *in vivo* activity. The *in vivo* activity was attributed to the active species **33**,

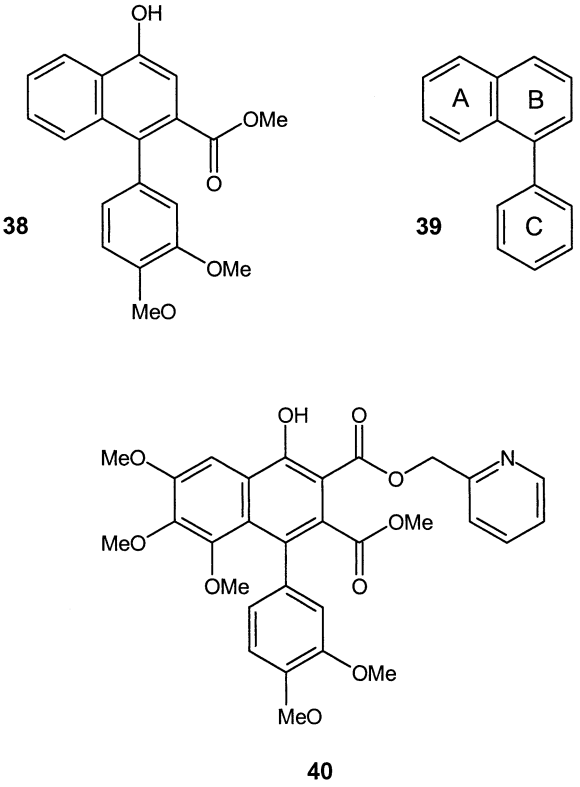


Figure 11. 1-Arylnaphthalene lignans as a lead for hypolipidemic agents (**38** – **40**)

by cyclisation of the open form **34** in the stomach or after absorption (Figure 10).

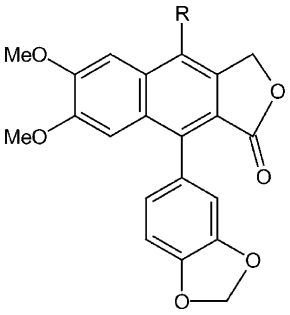
Unfortunately, compound **33** was found to be extensively metabolised *in vivo*, and synthetic derivatives were prepared to improve to metabolic stability (Delorme et al., 1996). Replacement of the lactone ring by a nitrile group, of the tetrahydropyran ring by a 6,8-dioxabicyclo[3.2.1]octanyl moiety (i.e., introduction of an additional methyleneoxy bridge), and of the pendant phenyl ring by a 3-furyl ring, led to the production of a potent and metabolically stable inhibitor of 5-lipoxygenase (**35**) (Figure 10). Compound **35** inhibited the oxidation of arachidonic acid by by recombinant human 5-lipoxygenase with an IC_{50} of, 190 nM), and the production of leukotriene B4 in human polymorphonuclear leukocytes (IC_{50} 3 nM) and in human whole blood (IC_{50} 150 nM). However, the replacement of the lactone moiety by a cyano group resulted in very lipohilic compounds that were generally poorly absorbed or not at all (Hamel et al., 1997). A strategy which was explored to improve the bioavailability was the replacement of the middle phenyl ring by nitrogen-containing heterocycles. These heterocycles could then be formulated as salts in order to improve aqueous solubility and oral absorption. In this way compound **36** was constructed, a potent inhibitor of 5-lipoxygenase, which could be absorbed orally. The IC_{50} values in the three *in vitro* systems mentioned above were 20 nM, 1.6 nM and 42 nM, respectively. The activity was confirmed *in vivo* in different animal models. Compounds where the naphthalene ring was replaced by a heterocycle, such as quinoline, were also prepared (Dubé et al., 1998). The 2-cyanoquinoline derivative **37** presented an overall biological profile similar to or better than the parent 2-cyanonaphthalene **36**, with IC_{50} values of 27 nM, 2.3 nM and 36 nM, respectively, and a good oral bioavailability and *in vivo* potency.

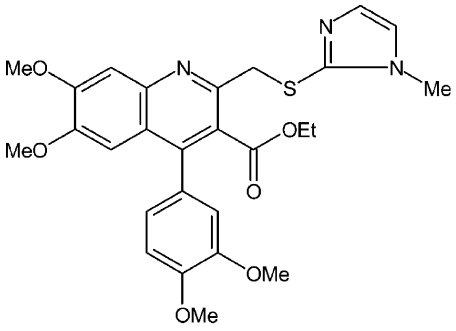
1-Arylnaphthalene lignans as a lead for hypolipidemic agents

In a series of synthetic 1-arylnaphthalene lignans, which were evaluated for their hypolipidemic activity in diet-induced hypercholesterolemic rats, compound **38** was initially selected for further evaluation, partly on the basis of structural novelty and ease of synthesis (Iwasaki et al., 1995). This lignan was able to lower total serum cholesterol while raising high-density lipoprotein cholesterol. It was active by inibiting intestinal absorption of both cholesterol and bile acids. The presence of the 1-(3,4-dimethoxyphenyl)-2-methoxycarbonyl-4-hydroxynaphhalene skeleton (**39**) was important for a high activity. The impact was explored of modifying the remaining ester functionality, and of introducing an heteroaromatic ring instead of ring A (Kuroda et al., 1997). In general, the heteroaromatic analogues were less active than the original lead. Modification of the C-3 ester functionality led to the design of the 2-pyridylmethyl ester **40** with optimal activity with regard to both hypocholesterolemic and HDL cholesterol-elevating properties.

1-Arylnaphthalene lignans as a lead for antirheumatic agents

Crude mixtures and semisynthetic derivatives of lignans from *Podophyllum emodi* have been used for the treatment of rheumatoid arthritis (RA) for many years. CPH82 is a mixture composed of two purified semisynthetic lignan derivatives, and has been shown to be clinically effective in RA patients (Larsen et al., 1989;

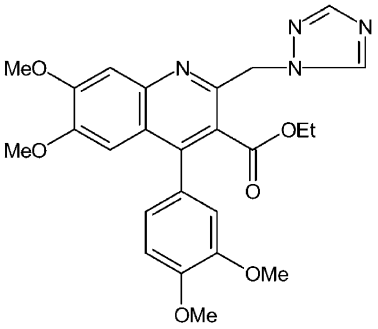


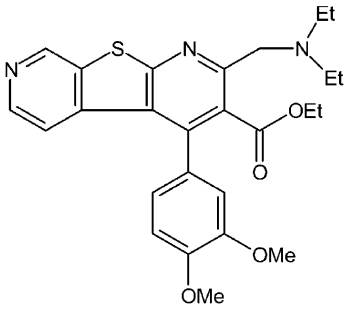


41 R = MeO

42 R = H

43





44 **45**

Figure 12. 1-Arylnaphthalene lignans as a lead for antirheumatic agents (**41** – **45**)

Truedsson et al., 1993). After two years of treatment of patients with early RA it was found to be as effective as methotrexate (Svensson and Pettersson, 2003).

Since the final stage of rheumatoid arthritis is bone destruction, the potent bone resorption inhibitors justicidin A (**41**) and B (**42**) were used as lead compounds for new drugs inhibiting bone resorption (Baba et al., 1996). Compound **43**, in which the naphthalene ring was replaced by a quinoline ring, and in which a methylimidazolyl moiety was introduced after opening of the lactone, was found to have anti-inflammatory properties in rats with adjuvant arthritis. Further modification of **43** lead to the design of **44** [ethyl 4-(3,4-dimethoxyphenyl)-6,7-dimethoxy-2-(1,2,4-triazol-1-ylmethyl)quinoline-3-carboxylate], a compound displaying a potent anti-inflammatory effect, which was selected as candidate for further investigation. Pharmacological investigation revealed that compound **44** had the profile of an immunomodulator, with the potential to control bone and cartilage destruction, but without any effect on prostaglandin biosynthesis. Structure-activity relationship studies with regard to the bone resorption inhibibitory effects confirmed that its activity was superior to the justicidins (Baba et al., 1999a). In a continuation of this work, the thieno[2,3-b : 5,4-c′]-dipyridine derivative **45** [2-(diethylaminomethyl)-4-(3,4-dimethoxyphenyl)thieno[2,3-b: 5,4-c′]-dipyridine-3-carboxylate] was found to be more potent than **44**. In general, the presence of the diethylamino moiety in the side chain was associated with a high anti-inflammatory effect (Baba et al., 1999b). Here too, the antiinflammatory activity could be attributed to

an immunomodulatory effect, without cyclooxygenase inhibiting activity.

Conclusion

In conclusion, it can be stated that the exploration of the chemical diversity of the naturally occurring lignans and neolignans has already resulted in the characterisation of many interesting lead compounds in various therapeutic areas; it is reasonable to expect that many more leads are still to be discovered and developed.

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