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# Chemoinduction of cytotoxic selectivity in Podophyllotoxin-related lignans

M.A. Castro*, J.M. Miguel del Corral, M. Gordaliza, M.A. Gómez-Zurita, P.A. García & A. San Feliciano

*Departamento de Química Farmacéutica, Facultad de Farmacia, Universidad de Salamanca, E-37007 Salamanca, Spain; *Author for correspondence (Tel: 34 923 294 528; Fax: 34 923 294 515; E-mail: macg@usal.es)*

*Key words:* cyclolignans, podophyllic aldehydes, podophyllotoxin derivatives, selective cytotoxicity, structural changes in the cyclolignan skeleton

**Abstract**

Lignans are widely distributed in the plant kingdom, and display a variety of biological activities which have attracted the attention of the scientific community for decades. Several representative compounds of the cyclolignan class, such as podophyllotoxin and its semisynthetic derivative, etoposide, are currently used for the clinical treatment of warts and malign neoplasms. Other cyclolignans are involved in antineoplastic and antiarthritic clinical trials. Numerous podophyllotoxin-related compounds have been prepared through modification of nearly all the positions on the cyclolignan skeleton in the search of new, more selective and less toxic anticancer drugs. Our group has been interested in the chemoinduction of drug selectivity for several years, and we have designed and prepared new podophyllotoxin derivatives by modification mainly on the C and D-rings of the podophyllotoxin skeleton. Those derivatives, bearing an electrophilic functionality at C-9, have shown, both *in vitro* and *in vivo*, a high degree of selectivity against colon carcinoma, and less cytotoxicity for other neoplastic systems and normal kidney fibroblasts. The main structural modifications found in the literature for the podophyllotoxin skeleton in the past decade, including those from our research group, are presented in this article.

## Introduction

Lignans are a large family of natural products characterized by the coupling of two phenylpropane units ($C_6$-$C_3$), they constitute a complex family of skeletons and characteristic functionalizations which can be subdivided into different groups, such as lignans, neolignans or oxyneolignans, among others (Moss, 2000).

Plants containing lignans have been used for centuries by many different cultures as folk remedies in the treatment of various diseases (Ayres and Loike, 1990); for example, as cathartics, poisons, antihelminthic or vesicant agents, and also in the treatment of rheumatoid arthritis or gastric ulcers, among other diseases (Ayres and Loike, 1990). The lignans present in these plants are often believed to be responsible for these therapeutic properties and the spectrum of activity for lignans is continuously being expanded with the discovery of new bioactive compounds or through the screening of different activities for already known compounds.

Podophyllotoxin (Figure 1), the main cyclolignan component of *Podophyllum* sp. resin, has been used as a cathartic, antirheumatic, antiviral, etc. Currently it is used clinically for the treatment of venereal warts caused by *Papilloma* virus (Beutner, 1996; Syed et al., 1995); however, its antitumour activity is the most attractive feature of its biological profile, and clinical trials were undertaken (Hartwell and Schrecker, 1958; Stähelin and von Wartburg, 1989). In spite of the severe gastrointestinal toxicity shown in these clinical trials, podophyllotoxin became a lead compound for the design of new drugs, searching for increased activity, decreased toxicity and, in general, improved pharmacological profile.

Thus, several hundred derivatives were prepared, culminating in the clinical use of some semisynthetic

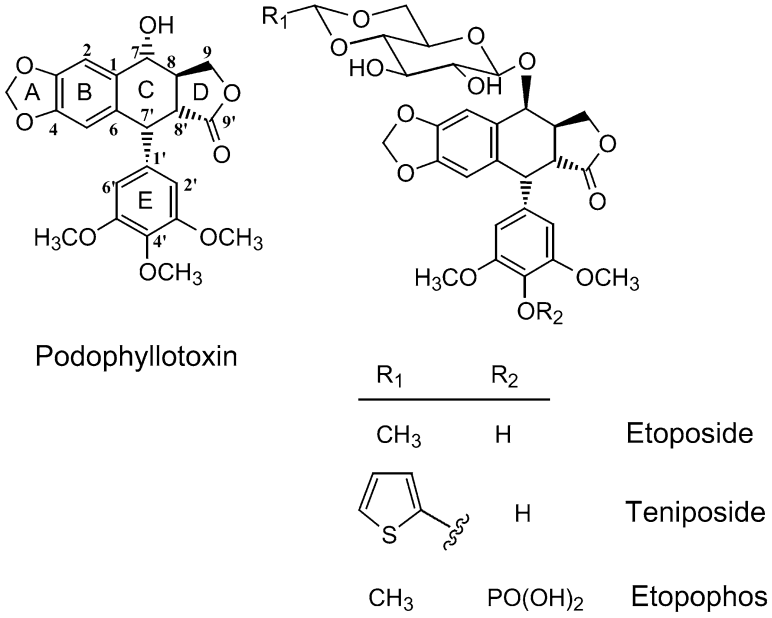



*Figure 1.* Structure and numbering of podophyllotoxin and its semi-synthetic derivatives in clinical use.

analogues named etoposide (Stähelin, 1973) and teniposide (Stähelin, 1970), developed in the late 60s and early 70s, and more recently etopophos (Hande, 1998; Schacter, 1996; Witterland et al., 1996), a more soluble prodrug of etoposide (Figure 1). Surprisingly, these semisynthetic derivatives and the parent compound podophyllotoxin showed different mechanisms of action (Jardine et al., 1980; Ayres and Loike, 1990).

Podophyllotoxin inhibits the assembly of tubulin into microtubules through interaction at the colchicine binding site (tubulin normally polymerises and gives rise to microtubules, which form the spindle during cell division); thus, when podophyllotoxin binds tubulin, cells are able to begin cell division with the prophase and the corresponding differentiation of chromosomes; however, those chromosomes are unable to separate because this process depends on the formation of the spindle. The result is that the cells, which have begun to divide, are arrested in the metaphase, remaining with their chromosomes joined together until they disintegrate some hours later.

Etoposide and related analogues are not inhibitors of microtubule formation. They induce a premitotic blockade in the late cell cycle S stage by binding to topoisomerase II, an enzyme required for the unwinding of DNA during replication. Topoisomerase II is assumed to form a transient, covalent DNA-protein link, the cleavage complex, which allows one double strand to pass through a temporary break into another double strand. Etoposide binds to and stabilizes the cleavage complex preventing repair of the double-stranded breaks.

These two mechanisms are not the only ones. There must be at least a third, undetermined, mechanism, because some derivatives have been reported to be as cytotoxic as podophyllotoxin and etoposide, but do not inhibit tubulin polymerisation and are only very weak inhibitors of topoisomerase II *in vitro* (Cho et al., 1996).

The main chemical modifications which convert podophyllotoxin from a compound that interacts with tubulin to one that inhibits topoisomerase II (Ayres and Loike, 1990) are: demethylation at C-4′, epimerization at C-7, glucosylation at OH-C-7, and acetalization of the 4- and 6-hydroxyl groups of the glucopyranose unit by aldehydes. Currently, these derivatives are widely used as anticancer drugs (stomach, ovarian, breast or small cell lung carcinomas among others) but they still have problems such as myelosuppression, poor bioavailability or drug resistance. Because of these problems, the aryltetralin lignans are still the subject of extensive research looking for new drugs with improved pharmacological profiles.

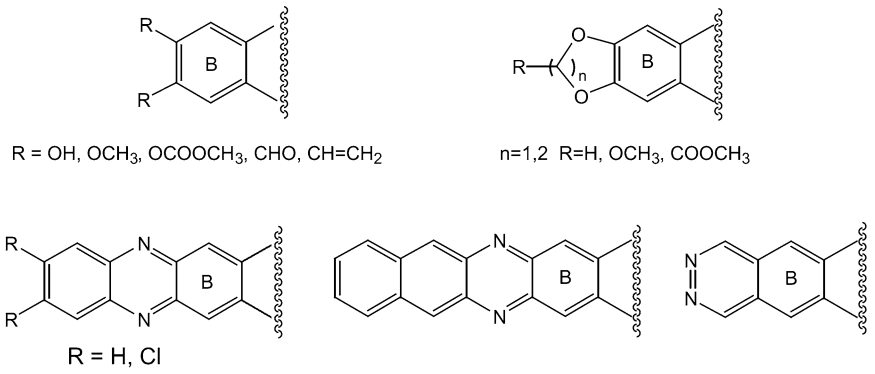



*Figure 2.* A-Ring modifications.

## Chemical transformations of podophyllotoxin

Considering the cyclolignan skeleton shown in Figure 1 for podophyllotoxin, every ring of the molecule (A to E) has been modified and the main chemical transformations reported during the last decade are referred to below; related compounds obtained by total synthesis are for the most part not considered. The changes reported in the literature and those performed by our research group are summarized on the basis of the ring modified, taking into account that it is also possible to find simultaneous changes on more than one ring.

### *Ring A*

One of the most common transformations performed on the A-ring (Figure 2) is the removal of the methylenedioxy group (Terada et al., 1992; Wang et al., 1992; Gordaliza et al., 1997a; Castro et al., 2003), keeping the two phenolic groups free or transforming them into ethers, esters, etc. (Maddaford and Charlton, 1993; Kamal et al., 1995; Bertounesque et al., 1996). Analogues with free phenols can bear diverse substituents at C-7. The dioxole ring has also been transformed into different rings such as substituted dioxoles or dioxanes (Castro et al., 2003b), phenazines (Cho et al., 1996) and phthalazines (Bertounesque et al., 1996).

The biological results indicate that the unsubstituted dioxole ring is important for the inhibition of both tubulin polymerisation and topoisomerase II, although the podophenazines showed high cytotoxicity values which must be produced through a third mechanism, as mentioned above (Cho et al., 1996). On the other hand, some derivatives lacking the methylenedioxy group, although much less cytotoxic than podophyllotoxin, are potent immunosuppressants (Gordaliza et al., 1997a).

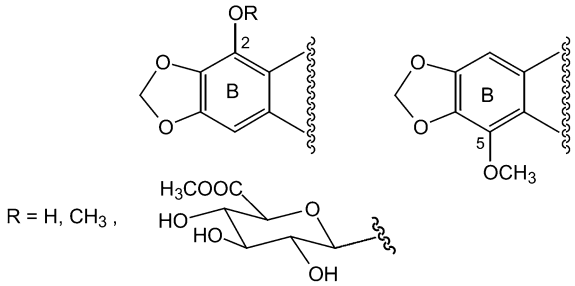



*Figure 3.* B-Ring modifications.

### *Ring B*

Few modifications have been performed on the B-ring (Figure 3). Such modifications are obviously directed to positions C-2 and C-5 and are usually related to the natural $\alpha$- and $\beta$-peltatins and 2- and 5-methoxypodophyllotoxins (Gu et al., 2002). Some of these compounds showed cytotoxicity values comparable to that of podophyllotoxin itself (San Feliciano et al., 1993; Gordaliza et al., 1994). Some glucuronides have also been prepared with the aim of obtaining less cytotoxic prodrugs, which would be hydrolysed in the tissues to the active species (Nudelman et al., 1997).

### *Ring C*

The C-ring is one of the most widely modified, with C-7 and C-8′ being the most important positions (Figure 4) to be changed. As mentioned before, the change of configuration of the C-7 substituent from $\alpha$ to $\beta$ gives rise to analogues with antitopoisomerase II activity and decreases the ability of the compounds to

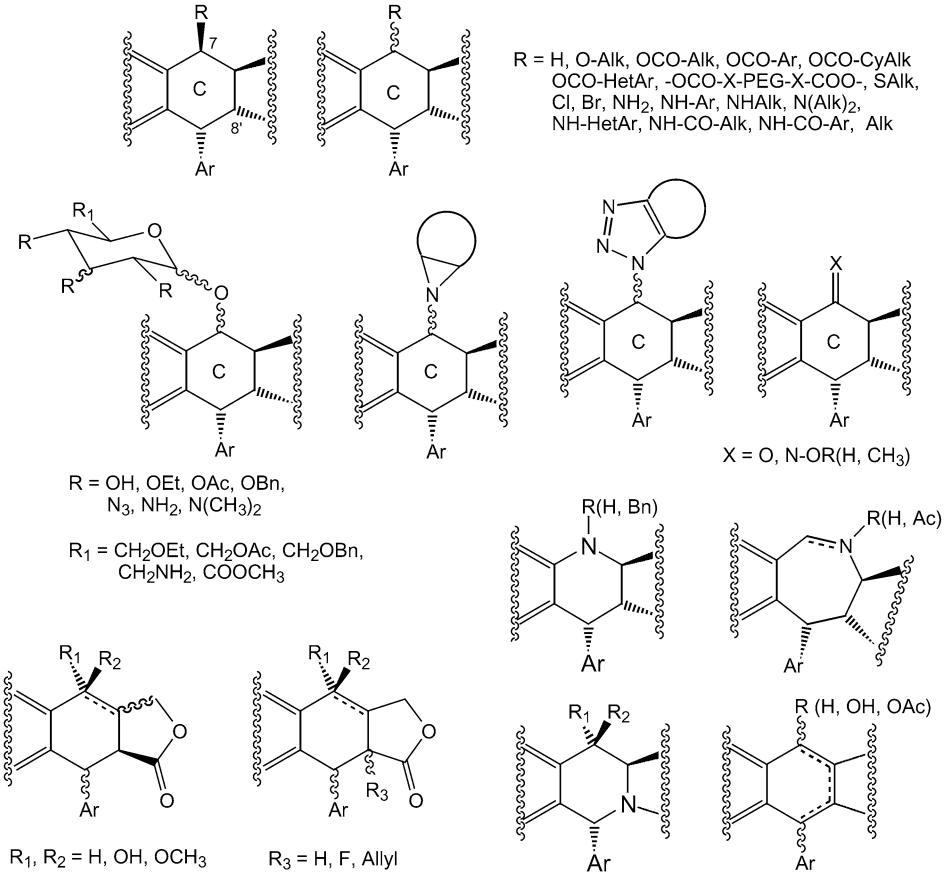



*Figure 4.* C-Ring modifications.

inhibit tubulin polymerisation. Many varied radicals have been introduced at C-7: ethers (Gordaliza et al., 1994; Miguel del Corral et al., 1995; Gupta et al., 1996; Wang et al., 1997b; Bathini et al., 1999), esters (Barro, 1996; Leav and Durst, 1996; Barajas, 1999; Greenwald et al., 1999; Lie Ken Jie et al., 1999; Wang et al., 2001), thioethers (Lee et al., 1997), alkylamino, arylamino and amido groups (Wang et al., 1997a; Damayanthi and Lown, 1998; Etievant et al., 1998; Zhu et al., 1999; Kamal et al., 2000; Shi et al., 2001), aziridines or triazoles (Laatsch et al., 1995), sugars (Hashimoto et al., 1991; Allevi et al. 1993; Nudelman et al. 1997; Daley et al., 1998), etc. Some directly attached alkyl chains have also been introduced at that position (Terada et al., 1993; Leav and Durst, 1996; Utsugi et al., 1996). In general, these modifications have been accompanied by demethylation at C-4′.

The cytotoxicity of several of these derivatives is comparable to etoposide and congeners. Other modifications of the C-ring include oxidation of the hydroxyl group to the corresponding ketone followed by its derivatization to give various oximes. In general, a loss of bioactivity is observed in these cases (Miguel del Corral et al., 1997).

Modification at C-8′ has been related to the metabolic inactivation of podophyllotoxin, since the *trans*-lactones are more potent than the *cis*-lactones (Gordaliza et al., 1995, 2000b). Hence, several substituents have been introduced (Zhou et al., 1994; Laatsch et al., 1996; Subrahmanyam et al., 1999; Van Vliet et al., 2001) in order to avoid or minimize that epimerisation, for example by introduction of fluorine (Van Vliet et al., 2001).

Formation of azaderivatives (Hitotsuyanagi et al., 1999), ring expansion (Laatsch et al., 1995) or aromatisation (Laatsch et al., 1995; Doré et al., 1996; Gordaliza et al., 2000b) are other modifications that concern the whole C-ring, but such modifications have never succeeded in improving the properties of the parent compound.

*Ring D*

The lactone ring has also been widely modified (Figure 5). Reports have mentioned introduction of some substituents (García, 2000), reduction to lactols or furans (Subrahmanyam et al., 1999; Roulland et al., 2000), opening of the lactone and further derivatization (Wang et al., 1993; Merino, 1995; Subrahmanyam et al., 1998; Tian et al., 2002), or formation of new rings such as the neoanalogues (Gordaliza et al. 2000a) or pyrazo and isoxazolignans reported by our group (Gordaliza et al., 2000b). The latter modifications lead to compounds that are less cytotoxic than podophyllotoxin, but are active as effective non-cytotoxic immunosuppressants *in vivo* (Gordaliza et al., 1997a).

*Ring E*

The trimethoxyphenyl ring has been related to active quinone metabolites generated through the *in vivo* oxidation of this type of drug (Van Vliet et al., 2001). Thus, the main modification reported is demethylation at C-4′, which can be seen to be very important for topoisomerase inhibition. The resulting phenol has also been further derivatized to easily-metabolisable groups such as esters or phosphates (Nudelman et al., 1997; Damayanthi and Lown, 1998; Canel et al., 2000) (Figure 6).

Other transformations imply changes in the degree of oxidation: catechols (Saulnier et al., 1993), orthoquinones (Zhang et al., 1992) and acetals (Pelter et al. 1993) have all been studied. All of these modifications led to less potent compounds than those of the 4′-demethyl series. Analogues without one, two or even all three methoxyl groups have been synthesized, in some cases with retention of the cytotoxicity (Berkowitz et al., 1996) of the parent compounds, which suggests that the three oxygenated functions are not a strong determinant of cytotoxicity.

Other analogues involving the formation of new heterocyclic systems, such as quinoxalines, phenazines or benzodioxanes, were prepared in our laboratory (Castro et al., 2003a). A general decrease in the cytotoxic potency was observed.

All these structural modifications have provided hundreds of podophyllotoxin analogues, most of which were several times less potent than the parent compound. However, it is worth mentioning that there are some derivatives which have reached phase I and phase II clinical trials (Figure 7) as antitumourals; this is the case for NPF (Daley et al., 1997), GL331

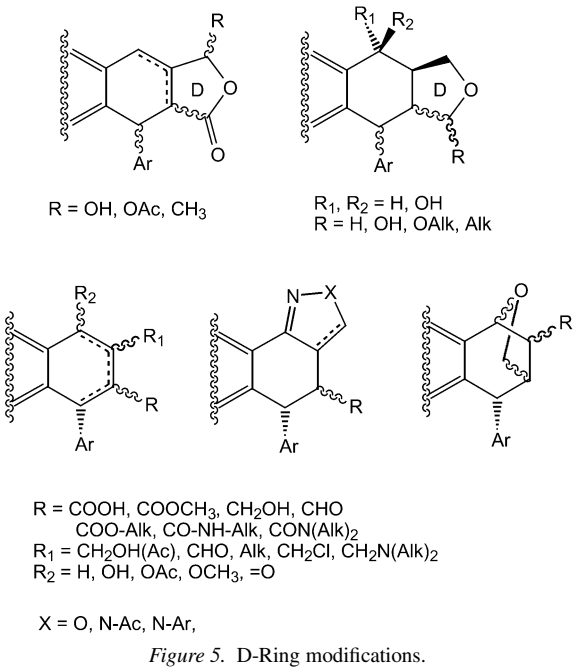



*Figure 5.* D-Ring modifications.

(Van Vliet and Lee, 1999), NK611 (Damayanthi and Lown, 1998), TOP-53 (Utsugi et al., 1996), and GP-11 (Wang et al., 1993). There is also a compound in a clinical trial as an antiarthritic (CPH-82) (Carlstrom et al., 2000).

## Preparation of selective cyclolignans

In our aim to obtain better podophyllotoxin-related drugs, our group has been involved for several years in the transformation of podophyllotoxin. We have prepared a large number of cyclolignans by modification of nearly all the rings of the skeleton, looking for more potent, less toxic and, preferably, more selective analogues. The plan and the preparation of selective cyclolignans are discussed below.

Eich et al. (1991) proposed a molecular mechanism for the interaction of etoposide with the cleavage complex (DNA-Topo II). According to this hypothesis, the free phenol at C-4′ would interact with DNA and the topoisomerase II would attack the carbonyl group at C-9′, suggesting that these lignans could be phosphorylated by a DNA phosphate group and could act as acylating agents for the topoisomerase. However, after studies with the stable conformers found for etoposide, we felt that this molecular mechanism looks improbable. The two bulky

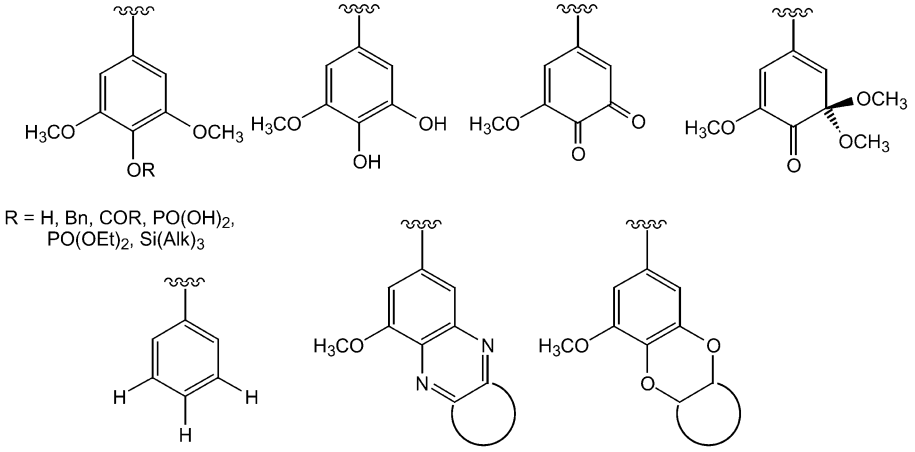
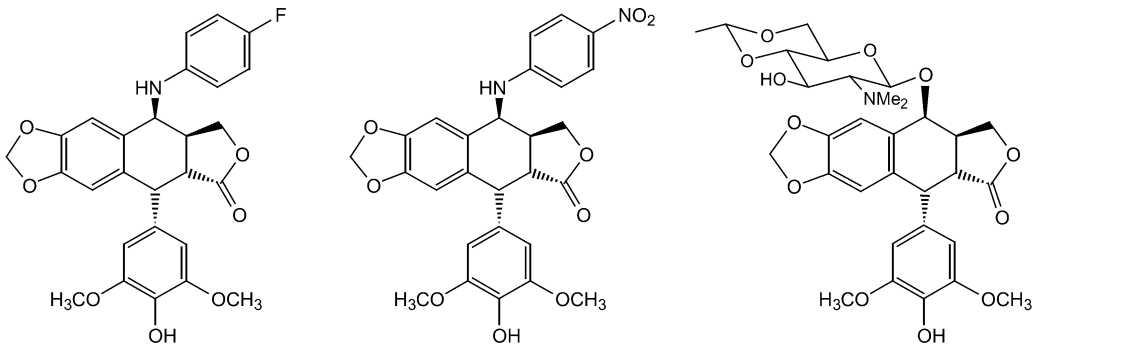



*Figure 6.* E-Ring modifications.



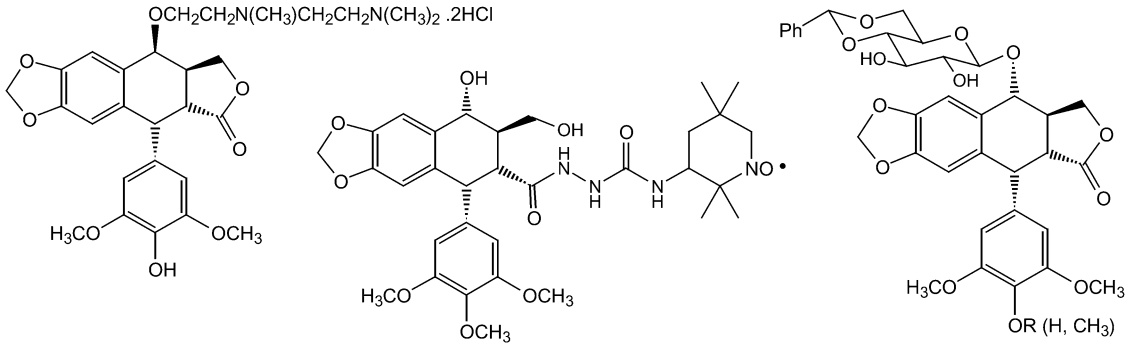
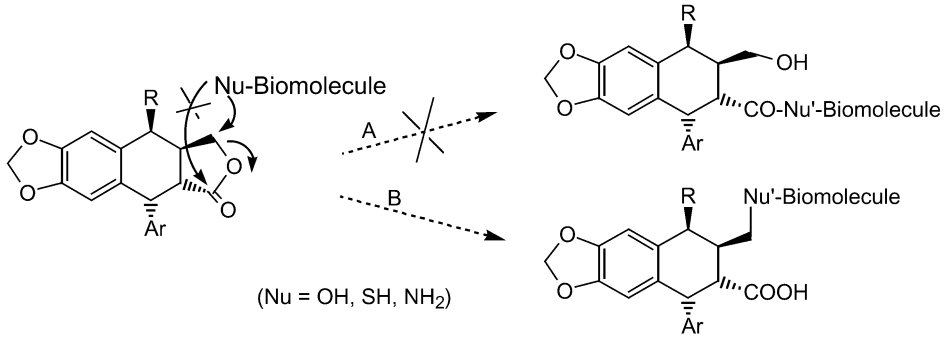
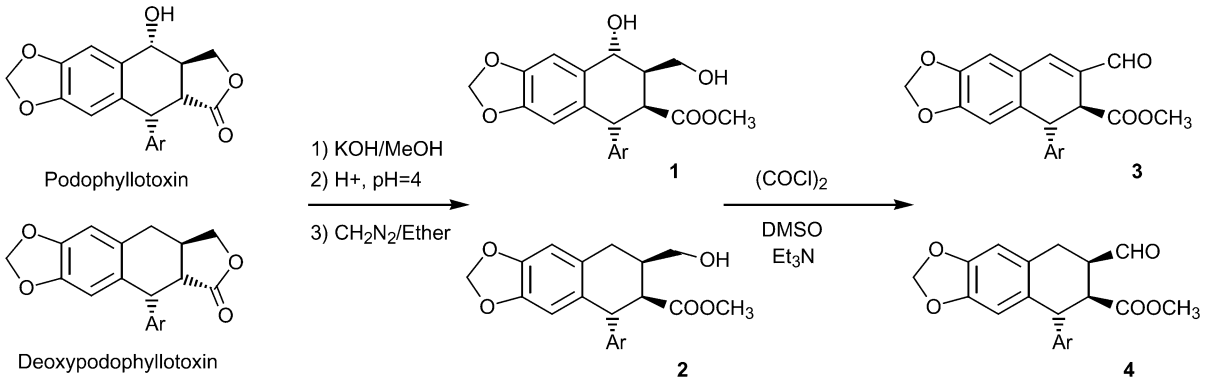




*Figure 7.* Several cyclolignans in clinical trials.




*Scheme 1.* Interaction of cyclolignanolides and biomolecules. A) Proposal for lignan lactones as acylating agents. B) New proposal for C-9 alkylating agents based on chemical evidence.




| Cytotoxicity (μM) | P-388 | A-549 | HT-29 | MEL-28 | SI |
|---|---|---|---|---|---|
| Podophyllotoxin | 0.012 | 0.012 | 0.029 | | 0.4 |
| Etoposide | 0.17 | 0.17 | 1.79 | 0.85 | 0.1 |
| Unsat. aldehyde **3** | 0.23 | 0.12 | **0.012** | 0.23 | 20 |
| Sat. aldehyde **4** | 2.34 | 2.34 | 2.34 | 2.34 | 1 |

[SI]=$IC_{50}$ (P-388) / $IC_{50}$ (HT-29)

*Scheme 2.* Podophyllic aldehydes. Preparation and cytotoxicity.

groups, sugar on the upper part and the pendant trimethoxyphenyl at the lower part, have almost free rotation, and thus it seemed to us that the approach of a large biomolecule to the C-9′ carbonyl of the lignans would be too hindered for an efficient acylation process. On the other hand, the electronically deficient C-9 methylene looks a more accessible position, which could be attacked by the enzyme from the rear side (Scheme 1). We thought that this could be the point of interaction with the biomolecule, whether for topoisomerase II or for biomolecules involved in tubulin polymerisation, and if this were to be the case, cyclolignanolides should be considered as alkylating agents. To further validate this idea, we confirmed chemically that weak nucleophiles were able to open the strained *trans*-lactone ring of podolignans (Gordaliza et al., 1995).

The ease with which the C-9 methylene reacts prompted us to modify the electrophilic character at that position, and we prepared two aldehydes from

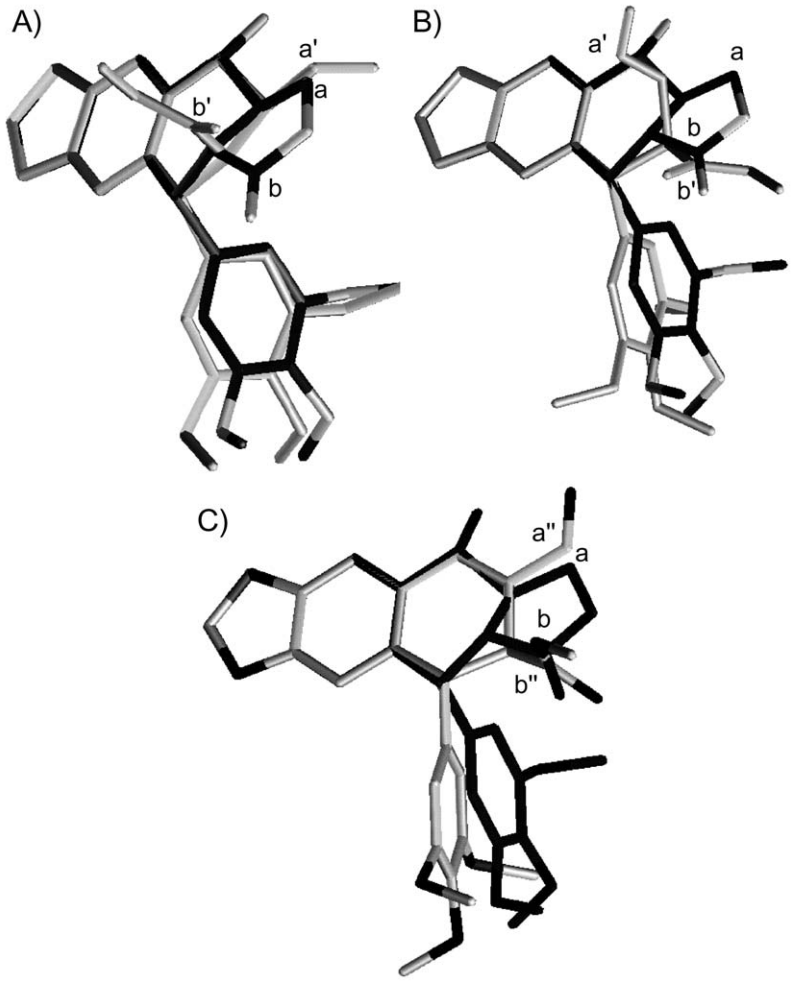
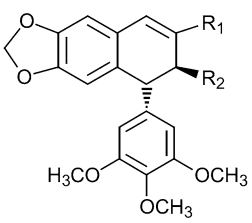


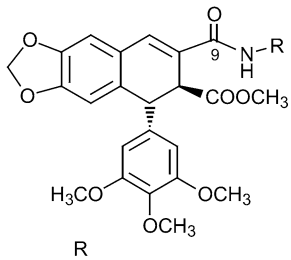

*Figure 8.* Superposition of podophyllotoxin and podophyllic aldehydes **3** and **4** based on the best fit for the tricyclic systems. A) Superposition of podophyllotoxin (black atoms) and **4** (grey atoms) matching C-9 carbons. B) Superposition of podophyllotoxin (black atoms) and **4** (grey atoms) matching C-9′ carbons. C) Superposition of podophyllotoxin (black atoms) and **3** (grey atoms).

podophyllotoxin and deoxypodophyllotoxin (Scheme 2). The lactone ring was opened under basic conditions and, after formation of the methyl esters **1** and **2**, the alcohols were oxidized under Swern conditions, yielding the two podophyllic aldehydes, **3** and **4**, differing only in the presence or absence of a double bond (Gordaliza et al., 1997b).

Both aldehydes were evaluated against four tumor cell lines and their cytotoxicity showed the relevance of that double bond. While **4** was somewhat less cyctotoxic than podophyllotoxin, **3** showed a very interesting selectivity index [SI] of 20 against colon carcinoma HT-29, with an $IC_{50}$ ($\mu$M) in the range of the *trans*-lactonic tetralins, which have always been reported as the most potent analogues (Ayres and Loike, 1990; Damayanthi and Lown, 1998; Gordaliza et al., 1994; Jardine, 1980).

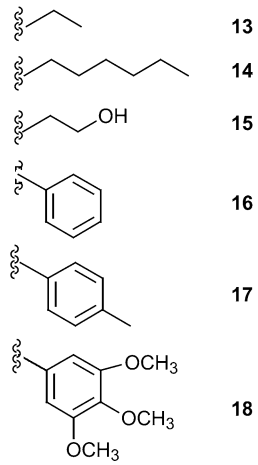
The differences in potency of these compounds cannot be explained by electronic factors (**4** is expected to be more electrophilic than **3** and should therefore be more potent). However, the presence of the double bond makes the molecule more rigid, and geometrically more similar to *trans*-lactones. This can be seen in the superposition of both aldehydes **3** and **4** with podophyllotoxin, based on the alignment of the tetracyclic ring system (Gordaliza et al., 1997b). Figure 8A shows the lowest energy conformation of **4** superimposed on podophyllotoxin using the ABC rings and C-9 carbons (a′, a) as matching points: as can be seen, the methoxycarbonyl group (b′) of **4** ap-

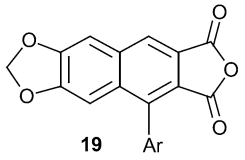


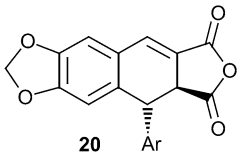



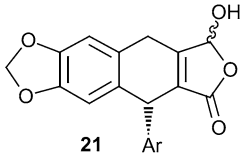

| | $R_1$ | $R_2$ | $IC_{50}$ (μM) P-388 | HT-29 | [SI] |
|---|---|---|---|---|---|
| **5** | CHO | $COOCH_3$ | 0.23 | 0.012 | [20] |
| **6** | CHO | $CH_2OH$ | 0.25 | 0.25 | [1] |
| **7** | CHO | CHO | 0.63 | 0.63 | [1] |
| **8** | $CH_2OH$ | $CH_2OH$ | 12 | 12 | [1] |
| **9** | $CH_2OH$ | $COOCH_3$ | 0.023 | 0.047 | [0.5] |
| **10** | COOH | $COOCH_3$ | >20 | >20 | [1] |
| **11** | COOH | COOH | >20 | >20 | [1] |
| **12** | $COOCH_3$ | $COOCH_3$ | 5.1 | 5.1 | [1] |

R



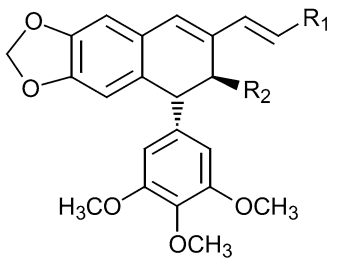

| | | | |
|---|---|---|---|
| P-388 | >20 μM | 12 μM | 1.2 μM | 1.65 -2.04 μM |
| HT-29 | >20 μM | 12 μM | 1.2 μM | 1.65 -2.04 μM |
| [SI] | [1] | [1] | [1] | [1] |

*Figure 9.* Podophyllotoxin analogues with different degrees of oxidation at C-9 and C-9′.

pears separated from the position of the lactone (b) of podophyllotoxin. If the C-9′ atoms (b, b′) are used as matching points, as in Figure 8B, the aldehyde group (a′) is now displaced relative to the corresponding atom (a) in podophyllotoxin. In contrast, both the ester and aldehyde functions of the unsaturated aldehyde **3** lie much closer to the corresponding groups in podophyllotoxin as can be seen in Figure 8C.

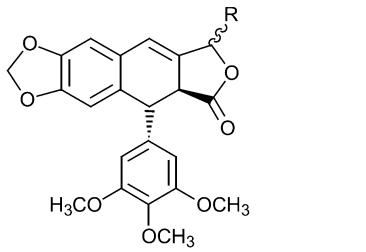
The podophyllic aldehyde **3** was submitted to the NCI for evaluation on its 60 tumour cell line panel and again the selectivity was observed for colon carcinoma cell lines (Log TGI (M) = −7.0) and also for CNS and breast cancers (Log TGI = −6.1 and −6.3, respectively). Apparently it is not a strong MDR substrate since the same potency was measured in strong MDR and non-MDR cell lines (Log TGI (M) = − 4.0). Nor did it affect mutant p53 cell lines and it blocked the cell cycle at the G2/M stage, thus indicating its ability to inhibit tubulin polymerisation and consequently displaying a mechanism of action similar to that of podophyllotoxin. The aldehyde **3** is also able to induce a delayed apoptosis characterized by caspase-3 activation, with a long lag between microtubule disassembly and the onset of apoptosis (Castro et al., 2004)

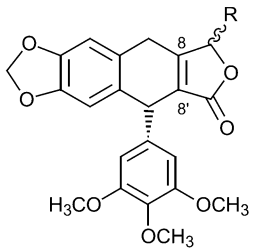
With these results in hand, the podophyllic aldehyde **3** seemed to us a good candidate for further manipulation and several transformations were carried out, trying to modify the electrophilic character at C-9, in order to analyze its influence on the selectivity observed for aldehyde **3**. Thus, the following modifications were considered: changes in the degree of oxidation of both C-9 and C-9′; formation of vinylogues; reactions with nucleophiles or synthesis of purinyl and other heterocyclic analogues. Most of the compounds prepared were evaluated against neoplastic cells and to provide an idea of their potency and selectivity the $IC_{50}$ ($\mu$M) for murine leukaemia P-388 and colon carcinoma HT-29 are included in the figures.



| | | | $IC_{50}$ (μM) | | |
|---|---|---|---|---|---|
| | $R_1$ | $R_2$ | P-388 | HT-29 | [SI] |
| **22** | CHO | $COOCH_3$ | 0.22 | 0.22 | [1] |
| **23** | $COCH_3$ | $COOCH_3$ | 0.27 | 0.27 | [1] |
| **24** | $NO_2$ | $COOCH_3$ | 0.27 | 0.27 | [1] |
| **25** | $COOCH_3$ | $COOCH_3$ | 10 | 2.1 | [5] |
| **26** | $COOCH_3$ | $CH_2OH$ | 2.6 | 2.6 | [1] |
| **27** | $CH_2OH$ | $CH_2OH$ | 2.3 | 2.3 | [1] |

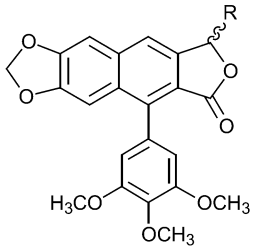
*Figure 10.* Vinylogues derivatives of podophyllic aldehyde.





| | | $IC_{50}$ (μM) | | |
|---|---|---|---|---|
| | R | P-388 | HT-29 | [SI] |
| **28** | H | 0.013 | 0.025 | [0.5] |
| **29** | α,β-$CH_3$ | 0.061 | 0.061 | [1] |
| **30** | α-$CF_2COOEt$ | 5.0 | 12 | [0.5] |

| | | $IC_{50}$ (μM) | | |
|---|---|---|---|---|
| | R | P-388 | HT-29 | [SI] |
| **31** | α,β-$CH_2CH{=}CH_2$ | - | - | |
| **32** | α-$CH_3$ | 0.12 | 0.024 | [5] |
| **33** | β-$CH_3$ | 0.024 | 0.024 | [1] |



| | | $IC_{50}$ (μM) | |
|---|---|---|---|
| | R | P-388 | HT-29 |
| **34** | H | 13 | >20 |
| **35** | α,β-$CH_3$ | >20 | >20 |

*Figure 11.* Analogues obtained by reaction of podophyllic aldehyde with C-nucleophiles.

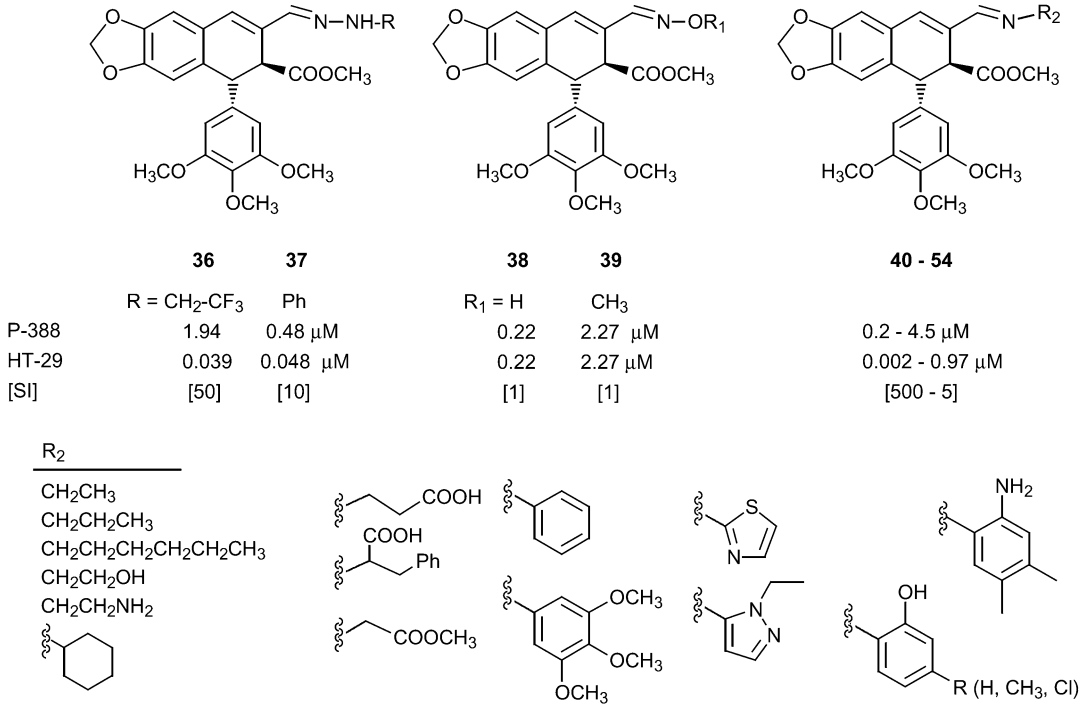



*Figure 12.* Analogues obtained by reaction of podophyllic aldehyde with N-nucleophiles.

*Different degrees of oxidation at C-9 and C-9′*

The degree of oxidation at both C-9 and C-9′ positions was modified by means of oxidation and reduction reactions. Several combinations of aldehyde-alcohol, carboxylic acid-ester, alcohol-ester, diesters, dialdehydes or diols at these positions were prepared (compounds **5-12**, Figure 9).

The $IC_{50}$ ($\mu$M) values for these derivatives indicated that the potency and the selectivity were lost except for the analogue **9** in which the potency was maintained, while the selectivity, if anything, was reversed, as occurred in podophyllotoxin. This result could be justified if, in the course of the assays, a relactonization could take place to give $\alpha$-apopicropodophyllotoxin **28**, which is as potent as podophyllotoxin (Gordaliza et al., 1994). The difference in the $IC_{50}$ for **5** and **6** seemed to indicate that it is not only the degree of oxidation at C-9 that is important, but also that at C-9′.

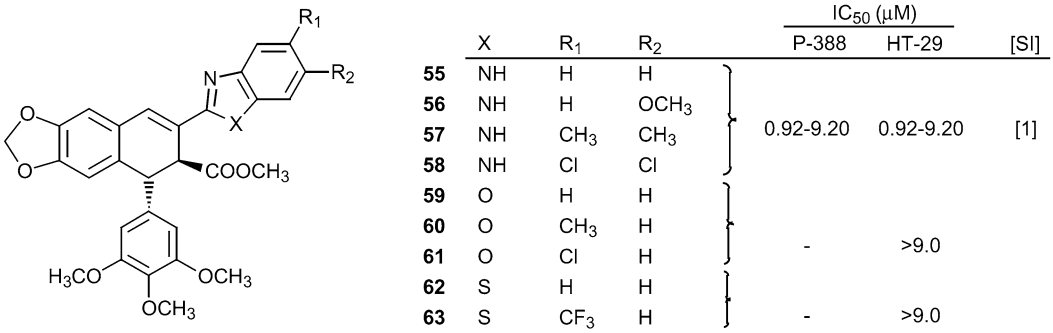
Those analogues with a higher degree of oxidation at C-9, such as **10** and **11** which bear a carboxylic acid, were nearly inactive. In some ways this was to be expected if we consider the postulated molecular mechanism discussed above. In such derivatives C-9 is less electrophilic, and to overcome that, some acid derivatives were prepared such as ester (**12**), anhydrides (**19**, **20**), amides (**13–18**) or lactol (**21**). Again the selectivity was lost, but amides and lactol partially recover the cytotoxicity if they are compared with the carboxylic acid precursor **10**. It can be deduced that not only the electrophilic character of the aldehyde, but also its accessibility (as a result of its practically free rotation) play an important role in the observed potency and selectivity.

*Preparation of vinylogues*

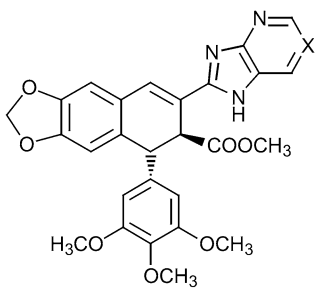
For the preparation of derivatives with an additional conjugate double bond, several Wittig reactions were performed and the extended aldehyde **22**, methylketone **23**, nitroderivative **24**, methylester **25** and allylic alcohols **26** and **27** were obtained (Figure 10). In all cases, most of the selectivity and the potency were lost.

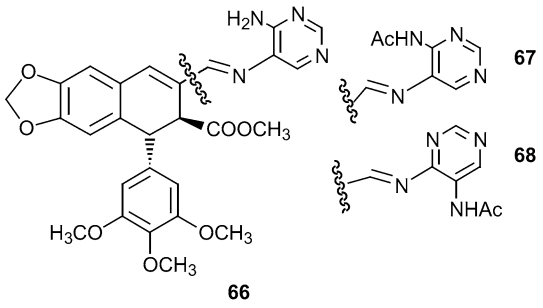
*Reactions with nucleophiles*

Making use of the electrophilic character of the podophyllic aldehyde, we synthesised several derivatives by reactions with carbon nucleophiles and nitrogen nucleophiles (Figures 11 and 12).




| | X | $R_1$ | $R_2$ | $IC_{50}$ (μM) P-388 | $IC_{50}$ (μM) HT-29 | [SI] |
|---|---|---|---|---|---|---|
| **55** | NH | H | H | 0.92-9.20 | 0.92-9.20 | [1] |
| **56** | NH | H | $OCH_3$ | | | |
| **57** | NH | $CH_3$ | $CH_3$ | | | |
| **58** | NH | Cl | Cl | | | |
| **59** | O | H | H | - | >9.0 | |
| **60** | O | $CH_3$ | H | | | |
| **61** | O | Cl | H | | | |
| **62** | S | H | H | - | >9.0 | |
| **63** | S | $CF_3$ | H | | | |




| | X | $IC_{50}$ (μM) P-388 | $IC_{50}$ (μM) HT-29 | [SI] |
|---|---|---|---|---|
| **64** | C | 1.90 | 0.5 | [4] |
| **65** | N | - | >9.7 | |

| $IC_{50}$ (μM) P-388 | $IC_{50}$ (μM) HT-29 | [SI] |
|---|---|---|
| 1.80-4.50 | 0.023-0.2 | [9-83] |

*Figure 13.* Purinyl, pyrimidinyl and other heterocyclic derivatives of podophyllotoxin.

As C-nucleophiles, Grignard and Reformatsky reagents were used. In all cases a relactonization was observed and, in some cases, after column chromatography on silica gel, the migration of the double bond to C-8-C-8′ was also observed, as in compounds **31–33**. In these compounds the tetracyclic system of podophyllotoxin is present and this is noticeable in the bioactivity: the potency and some selectivity were retained and the $IC_{50}$ values are in the range of podophyllotoxin. The size and the orientation of the new substituent at C-9 seemed to be important for selectivity towards the HT-29 carcinoma. The aromatic naphthalene derivatives **34** and **35** were less potent analogues, as previously published (Gordaliza et al., 1994; Doré et al., 1996).

As N-nucleophiles, substituted hydrazines, hydroxylamines and amines were used and the corresponding hydrazones **36** and **37**, oximes **38** and **39** and imines **40–54** were obtained. The imine series was the largest one, containing aliphatic chains, aminoacids, aromatic and heteroaromatic fragments.

As far as the $IC_{50}$ ($\mu$M) is concerned, the oximes lost selectivity but hydrazones and imines improved considerably upon both the potency and the selectivity of the parent podophyllic aldehyde, attaining an SI level up to 500 (the best result being obtained with the ethylamine derivative (Castro et al., 2004)).

*Synthesis of purinyl and other heterocyclic derivatives*

Some of the anilines used to obtain the imines bear another heteroatom (N, O or S) that can also act as a nucleophile and add to the double bond of the initially formed imine. Therefore, we decided to bring the process to completion to obtain the corresponding benzazoles (Figure 13): benzimidazoles **55–58** from phenylendiamines, benzoxazoles **59–61** from *o*-aminophenols and benzothiazoles **62–63** from *o*-aminothiophenols. All of the resulting compounds lost the selectivity and were less potent than their imine precursors.

Other heterocycles considered for attachment to the lignan structure were purines and pyrimidines, systems present in important endogenous compounds such as the nucleosides and in numerous antineoplastic therapeutic agents. Thus, diaminopyridine and diaminopyrimidine were used to obtain the corresponding purinyl derivatives **64–65**. If the reaction is stopped at the intermediate imine, the pyrimidinyl analogues **66–68** were obtained.

Regarding the observed $IC_{50}$ values, the pyrimidinyl analogues retained potency and selectivity, but the purine derivative did not. Work is in progress in order to introduce biological purines such as guanine or adenine.

In summary, starting from a natural product such as podophyllotoxin, we have prepared a cytotoxic and selective cyclolignan, the aldehyde **3**, lacking the $\gamma$-lactone ring generally considered an important feature for the bioactivity of podophyllotoxin analogues. Chemical transformations performed on this aldehyde have yielded derivatives in which not only the potency was improved but also the selectivity increased considerably in those analogues in which C-9 retained its electrophilic character, as in the imines **40–54**, confirming that the carbon C-9 is an important point in the interaction with biomolecules such as tubulin. While the great majority of work found in the literature is oriented towards inhibitors of topoisomerase II, studies performed with some of our derivatives indicated that they have the same mechanism of action as podophyllotoxin, that is, inhibition of tubulin polymerization.

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