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Synthesis and cytotoxicity of hydrophobic esters of podophyllotoxins

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Abstract—Diverse norbornenecarboxylate esters of podophyllotoxin and its epimers and diastereoisomers have been prepared through Diels–Alder cycloaddition by treating the dienophilic acrylates of cyclolignans with cyclopentadiene. Their cytotoxicities against several cancer cell lines have been evaluated and the results compared with those found for other lignan esters. Podophyllotoxin adducts showed a one-fold increase in activity when compared to the natural product. The preparation of more hydrophobic esters, which showed less cytotoxicity, demonstrated that this activity is not primarily due to the lipophilic factor, but mainly to the spatial arrangement of the bulky moiety, which could contribute to increase the binding to the target site.

Currently, etoposide (**1a**) is one of the most prescribed anticancer drugs, with good clinical perspectives against several types of cancer.[1] Etopophos (**1b**) is a new etoposide phosphate designed to overcome the limitations associated with the poor solubility of etoposide.[2] The introduction of these compounds into the market, is a good example of the development of therapeutically useful drugs derived from a natural product, such as podophyllotoxin (**2a**) in this case. NK611[3] (**1c**) and GL331 (**1d**) are two related derivatives which are presently under clinical trials.[4] GL331 has a promising potential in the treatment of gastric carcinoma, colon cancer and non-small-cell lung cancer.[5] **1a** and **1b**, two 4′-demethylepipodophyllotoxin derivatives, are potent inhibitors of DNA topoisomerase II, while podophyllotoxin acts by a different cytotoxic mechanism, primarily inhibiting tubulin polymerization through its interaction at the colchicine binding site (Fig. 1).

Despite intensive efforts, which include research for better podophyllotoxin derivatives, no substance has been found that can outperform the natural product in its efficacy for the inhibition of microtubule assembly.[6] In addition, podophyllotoxin and all of its related display the typical adverse effects common to most antineoplastics, inducing anemia, hair loss and severe gastrointestinal disturbances. Consequently, rather than more potent antimitotics of this type, it would be most interesting to discover more selective drugs with less adverse effects and, if possible, displaying an even better affinity for the colchicine binding site. These substances could lead to therapeutical agents that could work by tubulin polymerization inhibition or, much better, by inhibition of both tubulin and DNA–topoisomerase II. With this objective in mind, we have prepared several bulky esters of podophyllotoxin and epipodophyllotoxin with the aim of adding an extra binding group that could improve the affinity for the target.

It is widely accepted from structure–activity studies in the field of podolignans that the *trans*-lactones are more potent antineoplastics than the *cis*-lactones.[7] Nevertheless, we have prepared not only *trans*-lactones, but also several *cis*-lactone derivatives aiming to study the efficacy of the different epimers and diastereoisomers as chiral auxiliaries of dienophiles in the control of the stereochemistry during the Diels–Alder reaction (Scheme 1) used for the synthesis of these derivatives. The results of these chemical studies will be published in the near future.

Keywords: Podophyllotoxin; Lignans; Norbornenecarboxylate esters.
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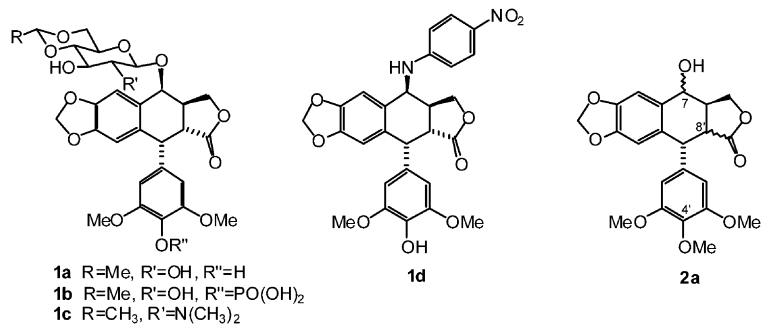
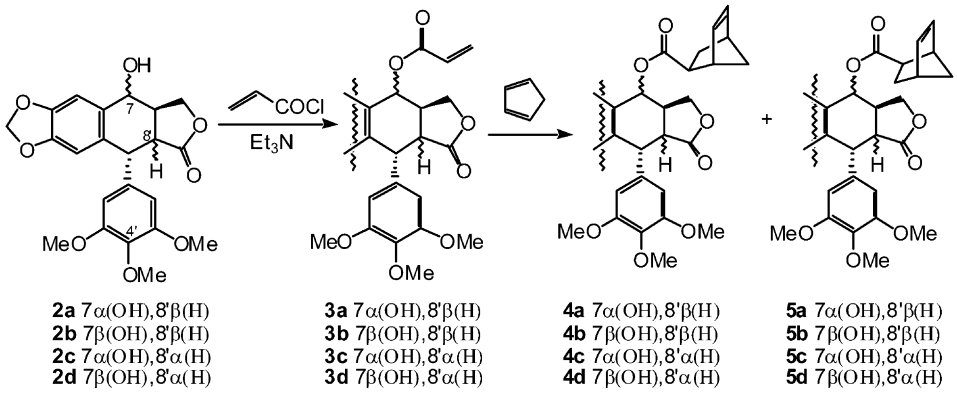


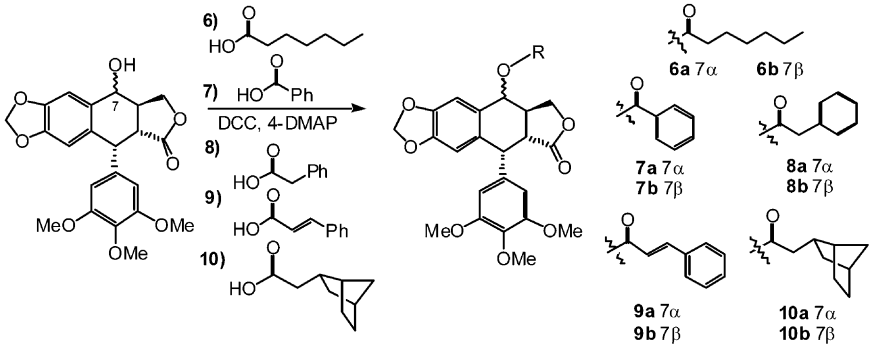
Figure 1. Lignans in the market or under clinical trials.



Scheme 1. Synthesis of acrylate and norbornenecarboxylate esters of podolignans.

In accordance with previously published results,[7] all *cis*-lactone derivatives described in this paper displayed less activity than the corresponding *trans*-lactones. The esters of podophyllotoxin[8] and epipodophyllotoxin[9] at the C-7 or C-4′ (previously demethylated) hydroxyl groups reported in the literature, are less potent than the parent lignan alcohols. Similar results were obtained by us when a number of similar derivatives were prepared (Scheme 2). When norbornenecarboxylates were obtained from the acrylate *trans*-lactone dienophiles, however, there was a one-fold increase in the cytotoxic potency with respect to the parent alcohols.

The synthesis of the proposed bulky lipophilic esters has been achieved through Diels–Alder cycloaddition of cyclopentadiene to several podophyllotoxin related acrylates as it is summarized in Scheme 1. The starting material **2a** was isolated from commercial podophyllin resin. The other three stereoisomers (**2b**, **c** and **d**) were obtained from podophyllotoxin by simple transformations: picropodophyllin (**2b**) by treatment of **2a** with methanolic KOH; epipodophyllotoxin (**2c**) by treatment with acid[10] and epipicropodophyllotoxin (**2d**) by treatment with acid followed by methanolic KOH. The acrylic esters (**3a**, **b**, **c** and **d**) were prepared by treatment of the corresponding alcohols with acryloyl chloride in the presence of triethylamine. The esters **3**, were then treated with cyclopentadiene affording the adducts **4** and **5**. Cyclopentadiene was prepared by cracking of dicyclopentadiene and used immediately.[11] It has been mentioned that the use of a Lewis acid catalyst in the Diels–Alder cycloaddition usually increases the rate of the *endo*/*exo* selectivity by controling the conformation of the transition state.[12] With this idea in mind, several Lewis acids were used, but no satisfactory results were obtained in any of the cases. When a recently distilled



Scheme 2. Acylation of podophyllotoxin and epipodophyllotoxin.

solution of BF_3OEt_2 was carefully added at −78 °C in an inert atmosphere, no reaction was observed, whereas if the temperature was slowly increased, an intense coloration appeared, giving place to a mixture of products that could not be resolved. When other catalysts such as $SnCl_4$, $TiCl_4$, $AlCl_3$ or $ZnCl_3$ were added at −78 °C, the reaction did not take place and it was necessary to increase the temperature up to 0 °C to obtain the desired adducts. In this case, however, no increase in the rate of the *endo*/*exo* selectivity was observed with respect to the reaction in thermal conditions. For this reason, all the cycloaddition reactions described in this work, were finally performed at −10 °C, in absence of catalyst.

The determination of the diastereomeric excess of the four Diels–Alder reactions was performed by HPLC analysis. The complete chemistry results will be published elsewhere. In the four cases, the two *endo* compounds were the major products while the two *exo* compounds appeared in proportion less than 0.5% of the crude reaction products. NMR studies of the diastereoisomeric pairs obtained from the four Diels–Alder reactions, showed that the major compounds were the *endo* adducts, in agreement with theoretical predictions. The mixture of the *endo* stereoisomers **4a** and **5a** was resolved by flash chromatography. The identification of every compound of each diastereoisomeric pair was achieved on the basis of specific rotation studies.[13]

The preparation of podophyllotoxin esters **6a–10a** was carried out with good yield, by treatment of podophyllotoxin, suspended in methylene dichloride, with the corresponding acid chloride (heptanoic, benzoic, phenylacetic, *trans*-cinnamic and norbornylacetic acids) in the presence of pyridine. Nevertheless, in the case of the preparation of epipodophyllotoxin esters (**6b–10b**), due to the axial disposition of the hydroxyl group, better results were achieved when using the Hassner[14] procedure, in which, epipodophyllotoxin is treated with the corresponding free acid (heptanoic, phenylacetic, *trans*-cinnamic and norbornylacetic) in presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (4-DMAP).

Cytotoxicities of all of the compounds prepared were tested in vitro following an adapted procedure of the method described by Bergeron[15] against different neoplastic cell lines: P-388 (suspension culture of a lymphoid neoplasm from DBA/2 mouse), A-549 (monolayer culture of human lung carcinoma), HT-29 (monolayer culture of human colon carcinoma) and MEL-28 (monolayer culture of human malign melanoma). The results of these assays were used to obtain the corresponding dose–response curves, from which IC_{50} (nM) values were calculated.

The results obtained are shown in Table 1. As it can be seen, the IC_{50} values of the majority of esters were either similar or better than those of the parent alcohols. Nevertheless, both podophyllotoxin norbornenecarboxylates **4a** and **5a** showed IC_{50} values about 4 nM, that represented an improvement in their potency with respect to the podophyllotoxin **2a**. The equipotency shown by compounds **4a** and **5a** against neoplastic cells is quite obvious and can be justified by their close similarity or identity in lipophilicity, configuration at C-7, global conformation and spatial distribution, in spite of the different configuration at position C-5 of the norbornene moiety, with the additional assumption of free rotation around the CO–norbornene bond.

Table 1. Cytotoxicity of lignans esters against four neoplastic cell lines. (Etoposide and podophyllotoxin as reference compounds. Data are expressed as IC_{50} nM values)

Compd	P-388	A-549	HT-29	MEL-28
1a*	170	170	1700	850
2a*	12	12	24	—
3a	21	21	21	21
4a	5	4	4	4
5a	5	4	4	4
6a	190	190	190	190
7a	24	48	48	48
8a	47	94	94	94
9a	184	184	184	184
10a	454	454	454	454
2b	60	60	60	—
3b	55	53	55	53
4b + 5b	19	19	19	19
7b	1930	1930	1930	1930
8b	188	188	188	188
9b	4591	9182	9182	9182
2c	6000	6000	6000	
3c	213	213	213	213
4c + 5c	1122	1122	1122	1122
2d	2900	2900	2900	—
3d	213	213	213	213
4d + 5d	468	468	468	468

In conclusion, the introduction of linear or aromatic acyl moieties at the C-7-OH of podolignans, generally induces either a loss of, or no effect upon, the cytotoxic activity of the parent hydroxylic derivatives. Nevertheless, when the acyl residue contains a norbornene fragment, almost a one-fold increase of activity is observed in the case of *trans*-lactones. This improvement of activity is more significant in the case of podophyllotoxin norbornene derivatives. The introduction of one additional methylene unit between the bicylic system and the carboxyl group (2-norborneneacetate of cyclolignan, **10a**) that would increase the lipophicity and the volume virtually occuped by the substituent, led to a diminished activity. These findings indicate that the main responsibility for the cytotoxicity change is not due to the lipophilic factor, but to the precise spatial arrangement of the bulky moiety, which may also contribute with additional non-polar interactions to enhance the binding to the target site.

Other compounds, mainly 4′-demethylderivatives will be prepared in order to clarify the structure–activity relationship for this family of compounds in relation with the DNA-topoisomerase II inhibition.

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