Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 21, pp. 2465-2468, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00432-7

SELECTIVE CYTOTOXIC CYCLOLIGNANS

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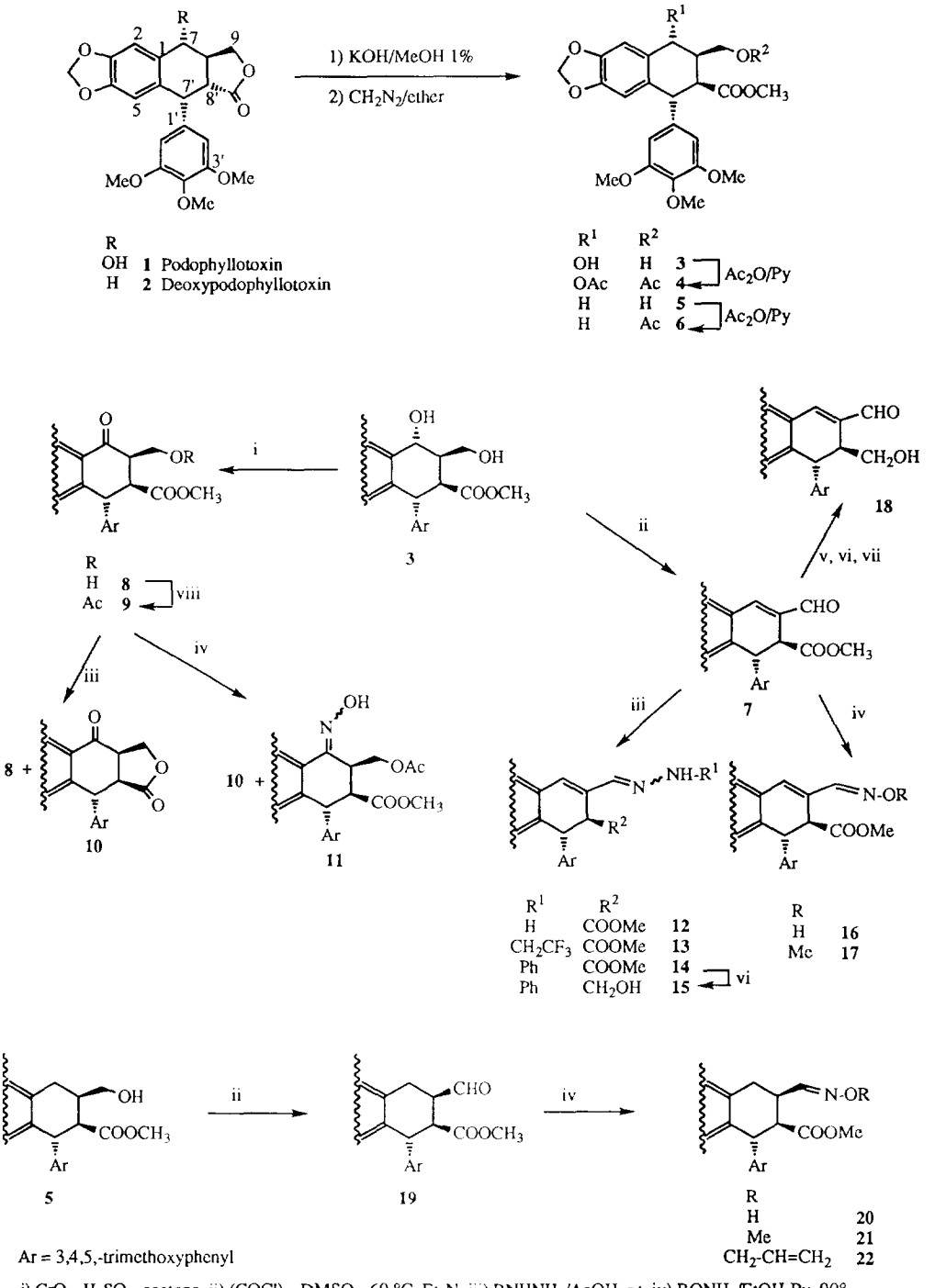
Abstract: Several derivatives of podophyllotoxin, lacking the lactone moiety and with nitrogen substituents at C-7 or C-9, have been prepared. They have been evaluated for their cytotoxic activity in P-388, A-549, HT-29 and MEL-28 culture cells. Some of them show a highly selective cytotoxicity towards HT-29 human colon carcinoma.

Etoposide and teniposide are two semisynthetic derivatives of podophyllotoxin which have played an important role in a wide variety of cancer chemotherapy protocols. Their use represents a therapeutic advance in the treatment of cancers, such as small-cell lung cancer, testicular cancer, lymphoma, acute lymphocytic leukaemia, etc.[1] However, the main deficiency of these compounds is their cytotoxicity for normal cells and the side effects derived from their lack of selectivity against tumoural cells. In this regard, it is necessary to investigate and prepare new analogues more potent and less toxic, that is, with better therapeutic indexes.

Several structure-activity studies on this field suggested that the presence of the lactone moiety is an important fact for displaying high cytotoxic activity, as well as the configuration at C-8'; it is well accepted that the *trans*-lactones are more potent as antineoplastic than the *cis*-lactones.[2] Many effort have been addressed to modify the lactone moiety and to prepare heteroanalogues at different positions of the cyclolignan skeleton.[3-5]

Over the last five years, our group has been carrying out studies on chemical transformations of podophyllotoxin and analogues and have prepared a large number of cyclolignan derivatives, some of which displaying potent anti-viral and cytotoxic activities.[6,7] More recently, we have reported a new class of cytotoxic agents, called pyrazololignans, which have nitrogen atoms at both C-7 and C-9. They are less potent than podophyllotoxin itself but with IC_{50} values at μM levels despite the lack of the lactone moiety in their structure.[8] In this sense and with the aim of getting more information about the influence of the heteroatoms in the structure-activity relationship, now we report other new cyclolignans also without the lactone ring and with only one nitrogen substituent at position C-7 or C-9 of the cyclolignan skeleton. They have been tested on cultures of different tumoural cell lines and have shown an interesting and selective cytotoxicity against HT-29 human colon carcinoma as it is discussed later in this paper.



Scheme 1. Preparation of hydrazones and oximes derived from podophyllotoxin.

Chemistry[9]

The starting compounds for all the reactions were podophyllotoxin **1** or its analogue deoxypodophyllotoxin **2** which were isolated from podophyllum resin.[10] Both were transformed into the methyl esters **3** and **5** by opening the lactone ring in basic conditions, which usually conduces to the epimerization at the C-8' position, leading to the most stable *cis*-junction between the lactone and the tetraline rings (Scheme 1).

Swern oxidation of diol-ester **3** yielded the unsaturated aldehyde **7**, while the oxidation with Jones' reagent led to **8** where only the benzylic hydroxyl at C-7 was oxidized. In the same way, Swern oxidation of **5** led to aldehyde **19**. The carbonyl compounds obtained were condensed with hydrazines or hydroxyl amines differently substituted as an easy method for the introduction of nitrogen substituents at positions C-7 and C-9.

The reaction between **7** and hydrazines in acetic acid led to the hydrazones **12-14**. The reaction with hydroxyl amines was made in ethanol and pyridine, leading to compounds **16** and **17**. The main reaction product of **8** or **9** with appropriate hydrazine was, instead the expected hydrazones, the ketolactone picropodophyllotoxone (**10**) which does not react with hydrazones in these conditions. The oxime **11** was obtained by condensation of **9** with the corresponding hydroxyl amine in low yield due to the formation of the by-product **10**.

In order to see the influence of different functional groups in the activity of these compounds, the methyl ester **7** was reduced, with the aldehyde previously protected as dithiane, by LAH to the hydroxy-aldehyde **18**. Similarly, the reduction of **14** led to the alcohol **15**.

Bioactivity

The prepared compounds have been evaluated for their bioactivity against cell cultures of P-388 murine leukaemia, A-549 human lung carcinoma, HT-29 human colon carcinoma and MEL-28 human melanoma. The cytotoxicity was performed over CV-1 monkey kidney fibroblasts.[11] The results obtained are shown in table 1.

Table 1. Antineoplastic activity of compounds **1-21**.(IC_{50} μM)

Compound	P-388	A-549	HT-29	MEL-28	CV-1
1	0.012	0.012	0.029		0.029
2	0.01	<0.006	0.006		0.006
3	0.22	0.22	0.45		0.45
4	1.1	1.1	1.1		1.1
5	0.058	0.058	0.12	0.058	0.058
6	0.21	0.21	0.21	0.21	0.21
7	0.23	0.12	0.012	0.23	0.23
8	5.63	5.63	5.63	5.63	5.63
10	12.0	12.0	12.0		48.0
12	0.57	0.57	0.57	1.14	2.27
13	0.48	0.19	0.048	0.48	0.48
14	1.94	0.97	0.039	4.84	1.94
15	1.02	1.02	2.05	0.51	1.02
16	2.27	2.27	2.27	2.27	2.27
17	0.22	0.22	0.22	0.22	0.22
18	0.25	0.25	0.25	0.25	0.63
19	2.34	2.34	2.34	2.34	2.34
21	10.94	21.88	>21.88	>21.88	>21.88

As it can be deduced from the data, most of the compounds shows similar responses for all the neoplastic systems tested except the compounds **7**, **13** and **14** which have been selective against HT-29 with selectivity index of 20, 10 and 50 respectively, if compared with their cytotoxicity against normal cells (CV-1). The IC_{50} of **7**, **13** and **14** against HT-29 is in the range of *trans*-lactonic cyclolignans which have always been the more potent analogues, any modification of the lactone moiety led to much less potent compounds.[6,7]

The fact that these compounds, even lacking the lactone moiety, are as potent as lactonic cyclolignans could be justified through a spontaneous hydrolysis of the hydrazones into the precursor aldehyde and this could be transformed in their lactonic analogue during the assays as it is shown in scheme 2.

Scheme 2.

The presence of the double bond between C-7 and C-8 makes the molecules more rigid, similarly to the *trans*-lactones where the four rings are almost coplanar and so that more reactive and easily opened by nucleophilic attack of several biomolecules.[8]

7 and **14** have also been tested by the NCI against 60 different types of cancers and they were selective for colon cancer and breast cancer according to the results obtained.

Acknowledgements:
National Cancer Institute Development Therapeutic Program.
Financial support for this work came from Spanish DGICYT (PB 93/616) and Junta de Castilla y León (Consejería de Cultura y Turismo, SA-35/94).

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11. Antineoplastic assays: Cells were seeded into 16 mm wells (multidishes NUNC 42001) at concentrations of $1x10^4$ (P-388), $2x10^4$ (A-549, HT-29 and MEL-28) cells/well, respectively, in 1 mL aliquots of MEM10FCS medium containing the compound to be evaluated at the concentrations tested. In each case, a set of control wells was incubated in the absence of sample and counted daily to ensure the exponential growth of cells. After three days at 37 °C, under a 10 % CO_2, 98 % humid atmosphere, P-388 cells were observed through an inverted microscopy and the degree of inhibition was determined by comparison with the controls, whereas A-549, HT-29 and MEL-28 were stained with crystal violet before examination.

(*Received in Belgium* 13 *June* 1995; *accepted* 18 *September* 1995)