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Laboratory note

Antitumor agents. Synthesis and biological evaluation of new compounds related to podophyllotoxin, containing the 2,3-dihydro-1,4-benzodioxin system

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Abstract – New compounds with naphtho-fused systems were synthesized and evaluated as antitumor agents. The naphtho-fused systems **6** and **7**, synthesized from the hydroxy-acetal, exhibit antitumor activity. The bis(phenylthio) derivatives were considered as possible precursors for lignan lactones (**11**). The hydroxy-naphthalen **6** showed a significant antineoplastic activity. © 2001 Éditions scientifiques et médicales Elsevier SAS

1,4-benzodioxin / naphtho-fused systems / antitumor agents

1. Introduction

The cytotoxic lignan lactone podophyllotoxin (**1**), the principal constituent of several plant species of Podophyllum, has attracted considerable interest in antitumor research [1, 2]. Although various chemical modifications of podophyllotoxin have been made, the function of the aromatic ring C on the activity have not yet been studied. Other lignan lactones [3] which have a biological significance are justicidine B (**2**) and the recently reported aza-analogue (**3**) [4] (*Figure 1*).

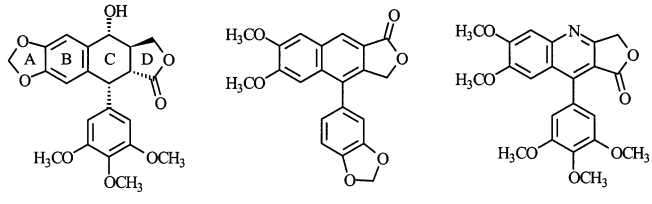

Fig. 1. Podophyllotoxin (**1**) and other lignan lactones with cytotoxic activity.

In view of the available information on structure–activity relationships, we decided to synthesize derivatives of **1** with a C-aromatic ring containing or not containing a hydroxyl group at the C-4 position. This led to the discovery of cytotoxic compounds avoiding stereochemistry problems.

2. Chemistry

The synthesis of the naphtho-fused systems **6** and **7** from *ortho*-disubstituted 1,4-benzodioxin intermediates in a one step reaction is shown in *Figure 2*. The hydroxy-acetal 4 was converted to the naphtho[2,3-b]dioxin systems by treatment with *p*-toluensulfonic acid or acetic acid and the monosubstituted isobenzofuran **5** formed in situ must be intercepted by a suitable dienophile (dimethyl acetylendicarboxylate or dimethyl maleate). Attempts to isolate the oxabicyclo adducts were not successful due to fast aromatization. All conditions studied for the formation of isobenzofuran, cycloaddition and aromatization are reported in a previous work [5].

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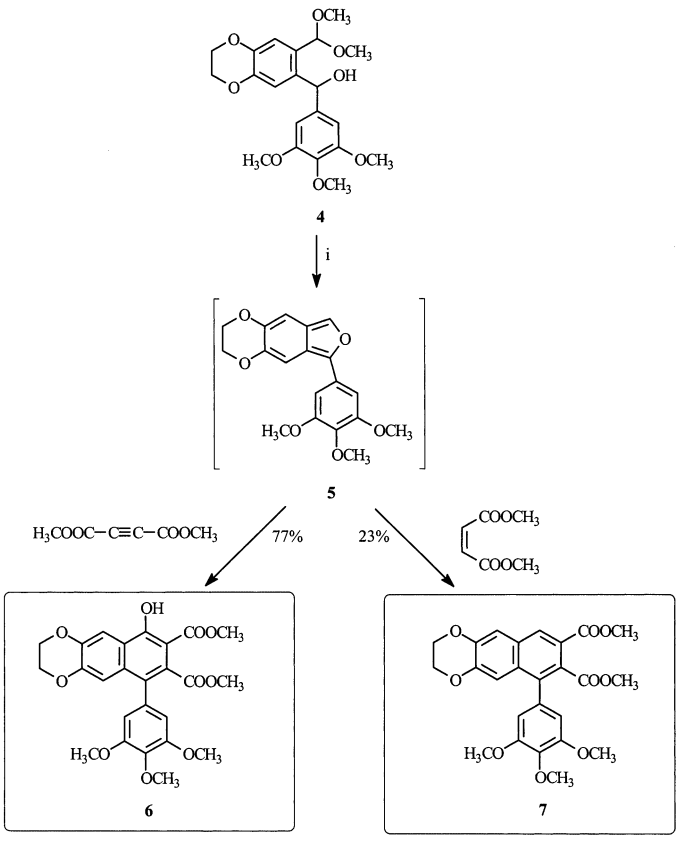

Fig. 2. Synthesis of the naphtho-fused systems **6** and **7** ((i) *p*-TSA or acetic acid).

The synthesis of new naphtho-lactone **11** is outlined in *Figure 3*. The butyrolactones **10a** and **10b**, are considered the key precursors for the synthesis of compounds **11** and **12**. Both compounds (**10a** and **10b**) were obtained through a tandem addition reaction of the dithiane anion of **9** to the 2(5*H*)-furanone and the 3,4,5-trimethoxybenzaldehyde [6]. The stereochemistry of the Cα and Cβ positions on the butyrolactone ring was assigned on the basis of the ^{1}H NMR spectral data by comparison with known compounds and it was assigned the *trans* stereochemistry indicated in the represented structure. Among the NMR data, we determined a value of $J = 14$ Hz between the protons linked to Cα and Cβ on the lactone ring in both of isomers (**10a** and **10b**), which confirms the assigned *trans* stereochemistry. Compound **10b** was assigned as the principal isomer by NMR spectra. The assignment was confirmed by analogy with related systems [7]. The selected protecting group of the corresponding aldehyde **8** was the *bis*(phenylthio) group, in order to make easy its subsequent elimination. However, the double presence of a benzylic group and the gem-diarylthio group in the same structure made impossible the following intramolecular cyclisation in the direction to obtaining the corresponding aryltetralin **12**. Several methods for the cyclisation were envisaged (*Table I*). It could be expected that the variation of the reaction conditions allowed direct the cyclisation at the formation of aryltetralin nucleus [8] (mild acidic) or the naphthalen nucleus [6] (heavy metal salts). Nevertheless, the high electronic density of the trimethoxyphenyl ring and the major reactivity of the carbocation in the phenylthio position allowed selectively the ring closure in the way to obtain the naphthalene nucleus (compound **11**) in good yield. The cyclisation using Lewis acid [9] was better than employing other acids. Thus, among the different tested conditions, a satisfactory yield of **11** was obtained when the intramolecular cyclisation was carried out with $SnCl_4$ in CH_2Cl_2 at room temperature during 10 min [6].

3. Results and discussions

These compounds were tested by National Cancer Institute *in vivo* and *in vitro*. All compounds were evaluated *in vitro* against a total of 60 human tumor cell lines derived from eight cancer types (leukemia, non-small cell lung cancer, small cell lung cancer, colon cancer, CNS-cancer, melanoma, ovarian cancer and renal cancer)[1]. The dose–reponse curves for each cell line were measured in all compounds with five different drug concentrations, and the concentration causing 50% cell growth inhibition (GI_{50}), total cell growth (TGI, 0% growth), and 50% cell death (LC_{50}, –50% growth) compared with the control was calculated. The $\log_{10} GI_{50}$ of compounds **6**, **7** and **11** as well as of **1** (podophyllotoxin) are expressed in the form of mean graphs (for compounds **6** and **7**, refer to *Figure 2*). In the graphs, the mean logarithmic value of GI_{50} in all cell lines for each tested compound is used as midpoint of that bar graph. Bars extending to the right represent sensitivity of the cell line to the test agent in excess of the average sensitivity of all tested cell lines. Bars extended to the left imply sensitivity less than the mean (are more resistant). The com-

[1] Conducted by the National Cancer Institute, Bethesda, MD, USA.

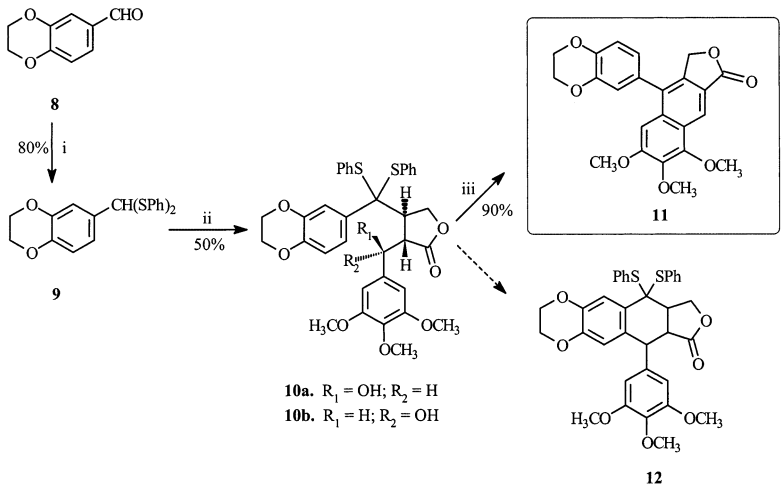

Fig. 3. Synthesis of new naphtho-lactone **11** ((i) PhSH–PTSA–toluene. (ii) 1. BuLi–THF; 2. 2(5*H*)-furanone; 3. 3,4,5-trimethoxybenzaldehyde. (iii) $SnCl_4$–CH_2Cl_2.

pound **6** has selectivity against leukemia, non-small cell lung cancer and renal cancer cell lines whereas the compound **7** shows selective cytotoxicity against CNS-cancer and melanoma. The compound **11** showed only weak activity (IC_{50} (L1210) $\approx 10^{-5}$ M; whereas the activity for etoposide is IC_{50} (L1210) = $0.12 \cdot 10^{-6}$ M) (*Figures 4* and *5*). These compounds **6** and **7** were selected for continuing the *in vivo* assays in the hollow fiber-based screen. Compounds which meet the Biological Evaluation Committee for Cancer Drugs (BEC/C) criteria for further testing are then referred for evaluations in subcutaneous human tumor xenograft assays. The related compound **6** was more cytotoxic than **7**, but both **6** and **7** showed a combined IP+SC score <20. (The criteria statiscally validated is IP+SC score $\geq$ 20.) The high cytotoxicity observed with compound **6** is probably due to the hydroxyl group at the C-4 position. These analyzed data suggested that the steric effects are influential for the antitumor activity, and the trimethoxy group of the compound **11** are in a position less favorable that the same group in the compounds **6** and **7**, the same problem is present in other series of compounds reported recently [4].

4. Experimental

All melting points were determined in capillary tubes on a Gallenkamp apparatus and are uncorrected. NMR spectra were recorded either on a Varian Gemini-200 MHz or/and Varian XL-300 MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from tetramethylsilane as the internal standard. Standard abbreviations are used to denote signal patterns. IR spectra were recorded in a FTIR Perkin–Elmer 1600 spectrometer. MS were performed on a Hewlett–Packard spectrometer 5988-A (70 eV). Elemental analyses were obtained from the Serveis Científico-Tècnics (Universitat de Barcelona). Reported analytical data are within ±0.4% of the theoretical values. Merck 60 (40–60 μm) and Merck 60 F_{254} silica gel were used for column chromatography and thin layer chromatography respectively. The organic extracts were dried over Na_2SO_4. Yields were not optimized.

*4.1. 2,3-Dihydro-1,4-benzodioxin-6-formyl-diphenylthioacetal (**9**)*

A solution of aldehyde **8** (1 g, 6.09 mmol), thiophenol

Table I. Attempts to cyclisation of **10**.

Entry	Reagents and conditions	Product	Yield (%) [a]
1	$BF_3 \cdot (CH_3CH_2)_2O$–CH_3NO_2/r.t./2 h	**11**	64
2	TFA–benzene–reflux/1 h	**11**	50
3	PTSA–toluene/reflux/24 h	**11**	50
4	Amberlist 15/CH_2Cl_2/r.t./24 h	**11**	40
5	$SnCl_4$–CH_2Cl_2/r.t./10 min	**11**	90

[a] Isolated compounds by column chromatography.

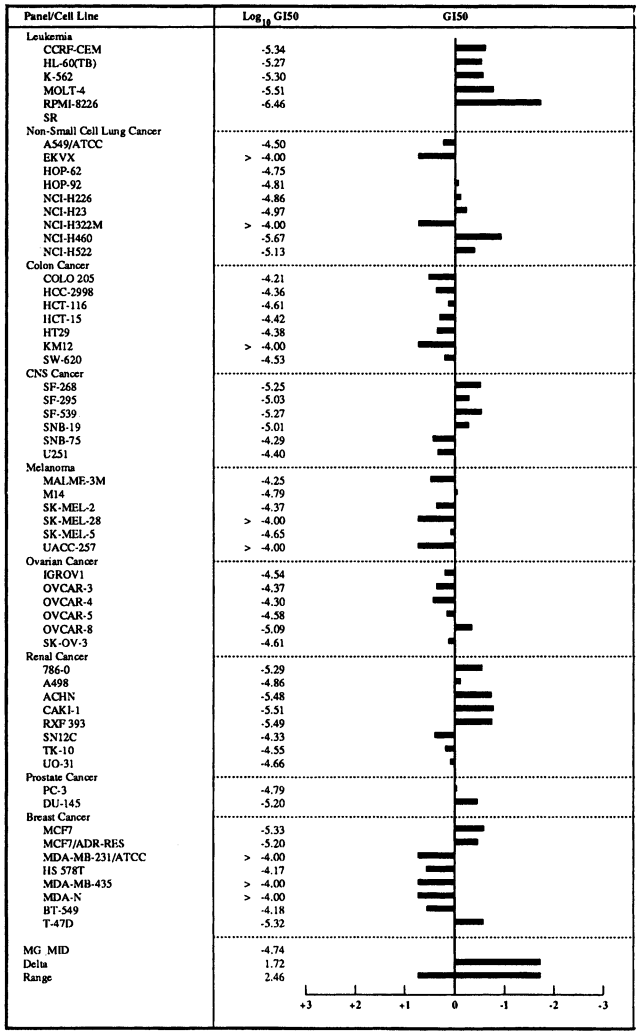

Fig. 4. In vitro activity-data of **6**. The numerical values listed are $\log_{10}$ TGI values which are the logs of the molar concentrations required for total growth inhibition. Bars projecting to the right on the mean bar graph indicate greater sensitivity, while these projecting to the left indicate less sensitivity.

(6.2 mL, 60.9 mmol, 1.077 g mL^{-1}) and a catalytic amount of PTSA in 30 mL of dry toluene was stirred at 140°C for 3 h. Then, the cooled mixture was extracted with ether (3×25 mL), dried and filtered. After removing the solvents under reduced pressure, the oil obtained was identified as the compound **9** (1.8 g, 80% yield). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 4.13 (s, 4H, CH_2O); 5.36 (s, 1H, $(PhS)_2CH$); 6.75–6.96 (m, 3H, C5–H, C7–H, C8–H); 7.17–7.37 (m, 10H, C2′–H, C3′–H, C4′–H, C5′–H, C6′–H). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 59.7 (CH, CHAr); 64.3 (CH_2, CH_2O); 116.7 and 117.1 (CH, C7, C8); 120.9 (CH, C5); 127.6 (CH, C4′); 129.0 (CH, C2′, C6′); 132.1 (CH, C3′, C5′); 132.8 (C, C1′, C6); 143.3 (C, C4a, C8a).

*4.2. trans-3-(3,4,5-Trimethoxyphenylhydroxymethyl)-4-[α,α-bis(phenylthio)-6-(2,3-dihydro-1,4-benzodioxinyl)methyl]butyrolactone (**10a** and **10b**)*

A suspension of diphenylthioacetal **9** (0.84 g, 2.3 mmol) and butyllithium (1.53 mL, 2.3 mmol) in dry THF (10 mL) was stirred at −78°C under an argon atmosphere. After 15 min, a solution of 2(5*H*)-furanone (0.2 g, 2.3 mmol) in 1 mL of dry THF was added and the mixture was stirred for 15 min. Then, a solution of

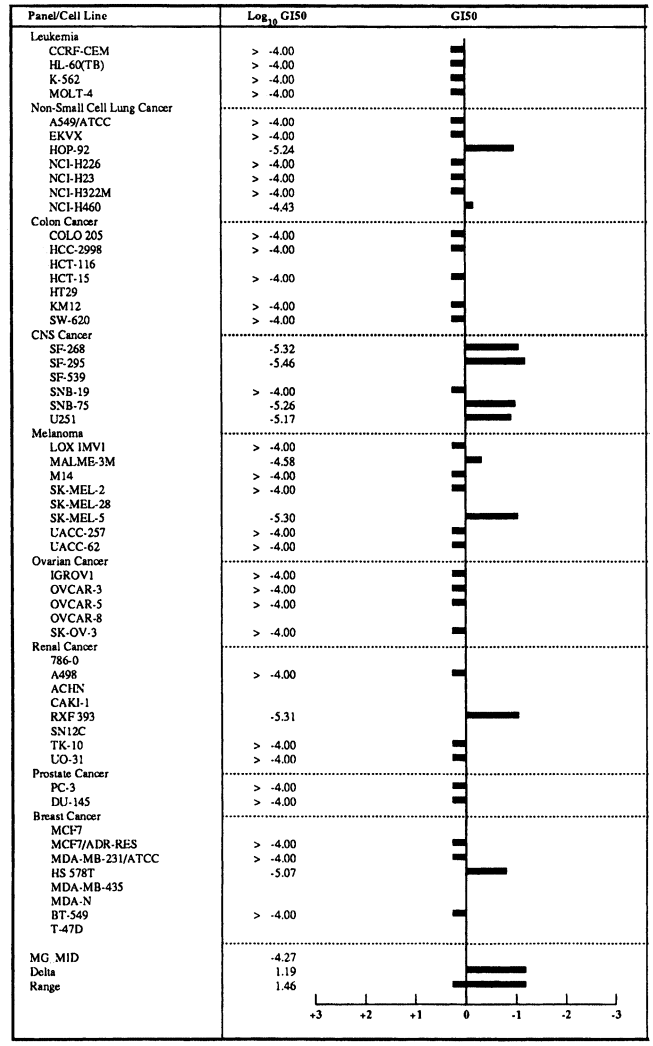

Fig. 5. Cytoxicity data of **7**.

3,4,5-trimethoxybenzaldehyde in dry THF (0.45 g, 2.3 mmol) was finally added. After stirring for 2 h, the mixture was hydrolyzed with a saturated solution of NH_4Cl and it was warmed to room temperature over night. When the solvent was removed under reduced pressure, the crude reaction was extracted with CH_2Cl_2 (3×20 mL), dried, filtered and concentrated under vacuo. The purification of the crude product by silica gel column chromatography (hexane–EtOAc: 50/50) afforded the enantiomeric mixture of alcohols **10a** and **10b** as a yellow solid (0.7 g, 50% yield). M.p.: 97–99°C (Hexane/EtOAc). Anal. Calc. for $C_{35}H_{34}O_8S_2$: C, 65.0; H, 5.3. Found: C, 64.8; H, 5.1%. MS (EI) (m/z): 646 (M^+), 198 ($C_{10}H_{14}O_4$). ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 2.78 (m, 1H, C_αHCO minor isomer); 2.85 (m, 1H, C_αHCO major isomer); 3.21 (t, $J=7$ Hz, 1H, C_βHCO major isomer); 3.42 (t, $J=7$ Hz, 1H, C_βHCO minor isomer); 3.75 (s, 3H, CH_3O); 3.79 (s, 3H, CH_3O); 3.83 (s, 3H, CH_3O); 4.24 (s, 4H, CH_2O); 4.62 (m, 2H, $COOCH_2$ minor isomer); 4.80 (m, 2H, $COOCH_2$ major isomer); 4.94 (m, 1H, CH–OH minor isomer); 5.15 (m, 1H, CH–OH, major isomer); 5.19 (bs, 1H, OH); 6.37 (s, 2H, C2′–H, C6′–H major isomer); 6.46 (s, 2H, C2′–H, C6′–H minor isomer); 6.91 (m, 1H, C5–H); 7.26 (cs, 12H, Ar). ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ: 45.7 (CH, C_αCO, major isomer); 46.1 (CH, C_αCO, minor isomer); 51.1 (CH, C_βCO, major isomer); 51.5 (CH, C_βCO, minor isomer); 56.1 (CH_3, CH_3O major isomer); 60.9 (CH_3, CH_3O minor isomer); 64.2 (CH_2, CH_2O major isomer); 64.3 (CH_2, CH_2O, minor isomer); 69.1 (CH_2, CH_2O, minor isomer); 69.8 (CH_2, CH_2O, major isomer); 72.8 (C, S–C–S, major isomer); 72.9 (C, S–C–S, minor isomer); 74.0 (CH, CH–OH, minor isomer); 74.1 (CH, CH–OH, major isomer); 102.8 (CH, C2′, C6′ major isomer); 103.0 (CH, C2′, C6′ minor isomer); 117.2 and 117.8 (CH, C5 and C8); 121.8 (CH, C7); 128.5 (CH, C2″, C6″); 131.5 (CH, C3″ and C5″); 132.9 (C, C6); 136.4 (CH, C4″); 137.9 (C, C1′); 138.9 (C, C1″); 143.4 and 143.6 (C, C4a, C8a); 153.3 (C, C3′, C4′, C5′); 176.1 (C, CO, minor isomer); 178.0 (C, CO, major isomer).

*4.3. 4-(2,3-Dihydro-1,4-benzodioxin-6-yl)-5,6,7-trimethoxy-1-oxo-3H-furo[3,4-b]naphthalen (**11**)*

To a solution of a mixture of compounds **10a** and **10b** (0.05 g, 0.08 mmol) in 5 mL of dry CH_2Cl_2, $SnCl_4$ (0.0084 mL, 0.08 mmol) was added and the suspension obtained was stirred, under an argon atmosphere at room temperature, for 10 min. Then, the mixture was poured into a saturated solution of $NaHCO_3$ (10 mL) and extracted with CH_2Cl_2 (3×15 mL). The combined organic layers were dried, filtered and concentrated. The crude product was purified by column chromatography on silica gel (hexane–EtOAc: 50/50) giving a white solid identified as the corresponding tricyclic compound **11** (28 mg, 90% yield). M.p.: 181–183°C (hexane–EtOAc). Anal. Calc. for $C_{23}H_{20}O_7$: C, 67.6; H, 4.9. Found: C, 67.4; H, 5.0%. MS (EI) (m/z): 408 (M^+). IR ($CHCl_3$) ν (cm^{-1}): 4213, 3619, 3012, 2421, 1762, 1420. ^{1}H NMR ($CDCl_3$, 200 MHz) δ: 3.38 (s, 3H, CH_3O); 3.93 (s, 3H, CH_3O); 4.03 (s, 3H, CH_3O); 4.34 (s, 4H, CH_2O); 6.80 (m, 2H, Ar); 6.92 (d, $J=10$ Hz, 1H, Ar); 7.20 (s, 1H, Ar); 8.30 (s, 1H, C9–H). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 55.9, 60.8 and 61.0 (CH_3, CH_3O); 64.4* (CH_2, C2′–H, C3′–H); 69.9* (CH_2, $COOCH_2$); 104.4 (CH, C8); 116.7 (CH, C5′, C8′); 121.0 (CH, C7′); 124.7 (CH, C9); 128.90 (C, C9a); 128.96, 129.0, 132.0, 132.7 and 139.1 (C, C4, C4a, C8a, C3a, C6′); 142.5 and 142.8 (C4′a, C8′a), 153.4 (C, C5, C6, C7); 171.8 (C, CO). *, interchangeables.

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