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# 9-DEOXOPODOPHYLLOTOXIN DERIVATIVES AS ANTI-CANCER AGENTS#

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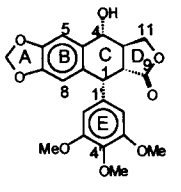
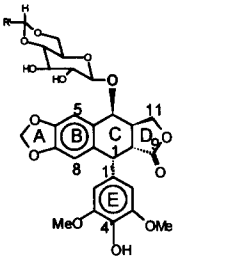
***Abstract :*** *Several 9-deoxo-9-substituted podophyllotoxin derivatives were synthesised starting from naturally occuring podophyllotoxin and their anti-cancer activity was evaluated against in vitro human cancer cell line assay. It was observed that these compounds do possess good anti-cancer activity particularly against ovarian, renal and lung cancer cell lines.* 

Podophyllotoxin **1** is a plant based lignan known to be highly cytotoxic towards various human cancers.[1] Structural modification of podophyllotoxin **1** led to the emergence of two less toxic, clinically useful anti-cancer drugs namely, etoposide **2** and teniposide **3**, which are presently used for the treatment of small cell lung cancer in humans.[2] However, to improve their clinical efficacy and reduce side effects[3], extensive structural modification of podophyllotoxin **1** has been pursued in various laboratories.[4] In particular, replacement of the $C_4$-OH group in **1** by a variety of substituents *via* oxygen,[5a] nitrogen[5a,b] and carbon[5c] linkages have been well studied in recent times.



**1**



**2** : R' = Me ; **3** : R' =

Most of these analogues prepared so far have the D-ring lactone intact, presumably because the *trans* fused -γ– lactone[6] was considered to be the strict structural requirement for their anti-cancer activity. Consequently, not many reports of 9-deoxo derivatives of podophyllotoxin **1** were found in the literature.[7] In continuation of our research interest in the preparation of semi-synthetic analogues of plant based pharmacophores,[8] we investigated the concept of replacing the carbonyl group of D-ring lactone in podophyllotoxin **1** with certain selected substitutents *via* oxygen and carbon linkages and evaluated their anti-cancer properties. To our surprise, most of these analogues were not devoid of their anti-tumor activity when tested against *in vitro* human tumor cell line assay. In fact, compound **13** showed good sensitivity towards most of the cell lines in an *in vitro* assay containing 20 human tumor cell lines. The synthesis and their *in vitro* anti-cancer acitivity of these 9-deoxopodophyllotoxin analogues is the subject matter of the present communication.

Podophyllotoxin **1** was reduced with diisobutylaluminium hydride (DIBAL-H) at -70°C to get the corresponding lactol **4**. Treatment of lactol **4** with ethyleneglycol in the presence of *p*-toluenesulfonic acid (PTSA) at 25°C produced 9-(2"-hydroxyethoxy)-9-deoxopodophyllotoxin **5**[9] in 60% yield, Scheme 1.

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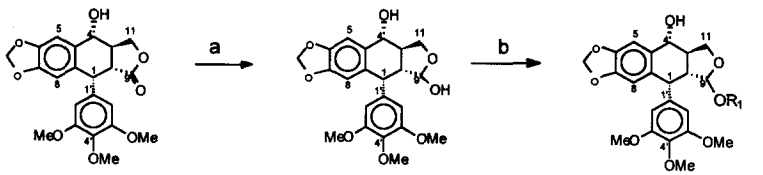
Similarly, treatment of lactol **4** with 2-methoxyethanol and 2-fluoroethanol under the influence of an acid furnished 9-(2"-methoxyethoxy)-9-deoxopodophyllotoxin **6** and 9-(2"-fluoroethoxy)-9-deoxopodophyllotoxin **7** respectively. .

**SCHEME 1**



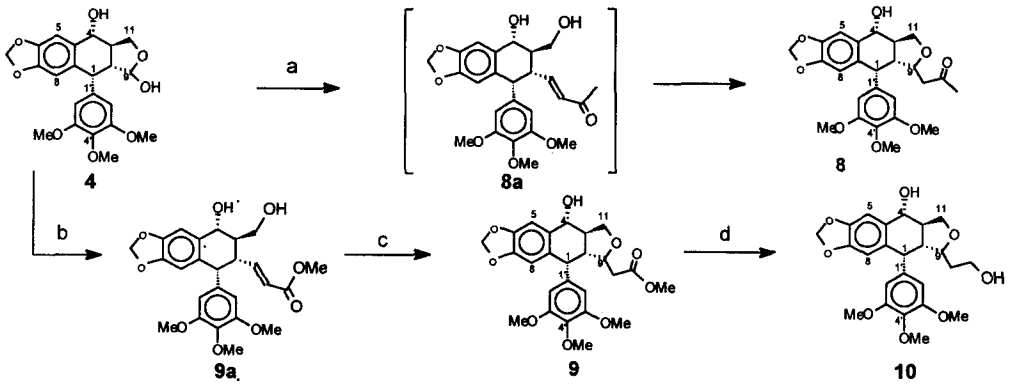
**1** **4**

**5**: $R_1 = CH_2\,CH_2\,OH$
**6**: $R_1 = CH_2\,CH_2\,OMe$
**7**: $R_1 = CH_2\,CH_2\,F$

**Reagents & Conditions :** (a) 1.0M soln.of diisobutylaluminiumhydride in toluene, DCM, -70°C, 40min., 70% ; (b) Ethylene glycol for **5**, 2-methoxyethanol for **6**, 2-fluoroethanol for **7**, 1,2-dichloroethane, *p*-toluenesulfonic acid, 25°C, 30min., 60-70% ;

To obtain the C-9-carbonsubstituted podophyllotoxin analogues, lactol **4** was treated with 1-triphenylphosphoranylidene-2-propanone in the presence of pyridine and the mixture was heated to 90°C in toluene to produce the compound **8** in 80% yield, Scheme 2. Reduction of the reaction temperature to 60°C only slowed down the production of **8** but the formation of the enone **8a** was not observed. However, treatment of lactol **4** with methyl (triphenylphosphoranylidene)acetate and pyridine at 90°C gave the α,β-unsaturated ester **9a**, which upon cyclisation using triethylamine produced the requisite ester **9**.[8] The compound **9** could also be obtained from **4** using pyridine / 110°C or triethylamine / 100°C. LAH reduction of the ester **9** furnished the corresponding diol **10**.[9]

**SCHEME 2**



**4** **8a** **8**

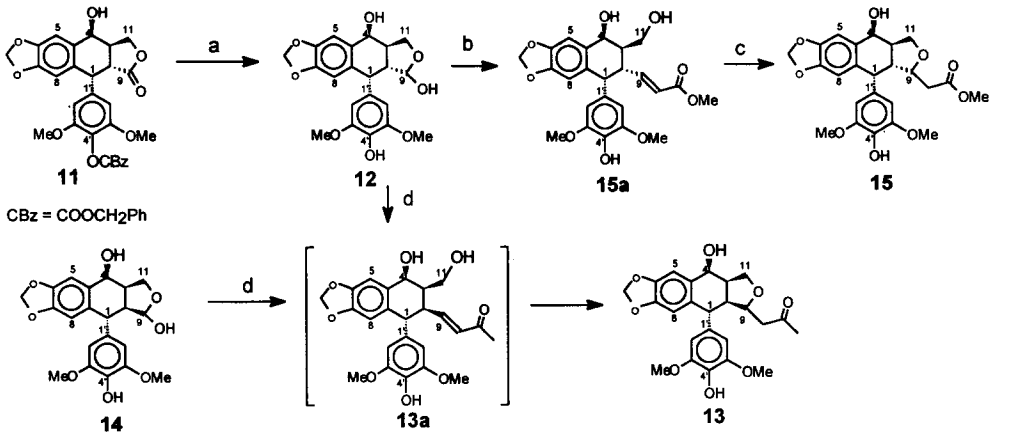
**9a** **9** **10**

**Reagents & Conditions** : (a) $PPh_3$=CHCOMe, toluene, pyridine, 90°C, 3h,80%; (b) $PPh_3$=CHCOOMe, toluene, pyridine, 90°C, 3h, 70%; (c) Toluene, $NEt_3$, 100°C, 80% ; (d) Lithum aluminium hydride, THF, 0°C, 1h, 80%;

Similarly, C-9 carbon substituted podophyllotoxin analogues having the free hydroxyl group at C-4'position were synthesised in the similar manner starting from 4'- demethyl-4'-O-(benzoyloxycarbonyl) epipodophyllotoxin **11**[10] as shown in the Scheme 3. However, in this case, the lactol **12** upon treatment with 1-triphenylphosphoranylidene-2-propanone, produced the corresponding picro derivative **13**.[9] The formation of **13** was also confirmed by its independent preparation *via* the conversion of picro lactol **14** under similar reaction conditions. Presumably, the epimerisation of the ring junction proton (C-2) in **13a** could be facilitated by the double activation due to the free phenolic hydroxyl as well as α,β-unsaturated enone moieties under the basic reaction conditions which led to the picro derivative. Incidentally, as expected, the corresponding ester derivative **15** was prepared from the lactol **12** without any epimerisation,

in good yield as shown in the Scheme 3. All these compounds represent mostly the single diastereomer in which the C-9 stereochemistry has not been established.

**SCHEME 3**




**Reagents & Conditions** :(a) 1.0M soln. of diisobutylaluminiumhydride in toluene, DCM, -70°C, 40min., 70%; (b) $PPh_3$=CHCOOMe, toluene, Pyridine, 90°C, 6h; (c) Toluene,$NEt_3$,100°C, 16h, 70%; (d) $PPh_3$=CHCOMe, toluene, pyridine, 100°C, 80%;

***In Vitro*** **Cytotoxicity :** The compounds were tested at our in-house facility against a panel of 7 human cancer cell lines taking one cell line from each cancer subtype following NCI's *in vitro* assay protocol.[11] The *in vitro* cytotoxicity (GI50) data of these compounds was presented in the Table 1. The data indicates that these compounds are not completely devoid of activity due to the lack of crucial lactone carbonyl group in their molecular framework. In fact some of these compounds have shown better activity than etoposide in certain cell lines. In particular ovarian, renal and lung cancer cell lines have shown more sensitivity towards these compounds. To our surprise, compared to the *trans* -C,D ring fused compounds the *cis* fused compound **13** showed equipotent activity to podophyllotoxin **1** in most of the cell lines tested, Table 1.

**Table 1** : *In vitro* cytotoxicity data of 9-deoxopodophyllotoxin derivatives :

| COMPOUND | CANCER CELL LINES | | | | | | |
|---|---|---|---|---|---|---|---|
| | SK-OV3 | MCF7-ADR | DU-145 | H 522 | M 14 | A 498 | SW 620 |
| 5 | **0.09** | 70 | 20 | 10 | 60 | **0.01** | 70 |
| 6 | 20 | >100 | 7.0 | **0.09** | >100 | **0.05** | 80 |
| 7 | >100 | 50 | 80 | 80 | 60 | 80 | 70 |
| 8 | **6.0** | 60 | 20 | 20 | 20 | **0.02** | 60 |
| 9 | **4.0** | 70 | 60 | **8.0** | >100 | **<0.01** | 70 |
| 10 | **0.8** | >100 | **0.5** | **0.05** | >100 | **0.05** | >100 |
| 13 | **0.3** | **0.8** | **0.1** | **0.8** | **0.8** | **0.08** | **0.1** |
| 15 | **0.5** | 60 | >100 | **9.0** | >100 | >100 | 20 |
| 1 | 0.9 | 0.6 | 0.04 | 0.7 | 0.07 | 0.08 | 2.0 |
| 2 | < 0.01 | 25 | 2.0 | 0.9 | <0.01 | 8.0 | 90 |

All the above values refer to GI50 given in μM concentrations. The term GI 50 stands for the concentration of the compound that produced 50% growth inhibition (**GI50**) in that particular cell line tested. Representative human cancer cell lines are Ovarian(SK-OV3), ADR resistant Breast cancer(MCF7-ADR), Prostate (DU-145), Lung(H 522), Melanoma(M-14), Renal(A-498) and Colon cancer (SW 620).

These results suggests that the D-ring lactone moiety in podophyllotoxin **1** may not be an essential feature for its anti-cancer activity. Moreover, the excellent activity shown by the compound **13** in comparison to other derivatives in the table 1 suggests that the trans fusion of C, D rings is also not a prime criteria when a suitable substitution at C-9 carbon is present in the ring D. Evaluation of these compounds for their pharmacokinetic study and the possible mechanism of action for the retention of cytotoxicity is in progress.

In summary a number of 9-deoxoderivatives of podophyllotoxin were prepared semi synthetically starting from the natural podophyllotoxin **1** and determined their *in vitro* anti-cancer activity in human cell line assay. Based on this study it was established that the non-lactonic podophyllotoxin derivatives also showed potential anti-cancer activity.

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**References and Notes :**

# Part of this work has been presented at the Eighth Symposium on the Latest Trends in Organic Synthesis -"Synthetic Pathways" held at Gainesville, Florida,USA during 28th Oct - 1st Nov'98.

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**9)** All the compounds have the satisfactory spectral and anlytical data. Spectral data of some selected compounds is presented here : **Compound 5** :mp : 135°C; IR : 3419, 1588, 1505, 1482, 1235, 1127, 935 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 200MHz) : δ 7.13(s, 1H), 6.45(s, 1H), 6.23(s, 2H), 5.96(s, 1H), 5.95(s, 1H), 4.70-4.55(m,2H), 4.33-4.20(m,2H), 3.95-3.60(m,5H), 3.86(s,3H), 3.78(s,6H), 3.05(br s, 1H, $D_2O$ exchageble), 2.48-2.33(m,2H), 2.00(br s, 1H, $D_2O$ exchangeble); $^{13}C$ NMR($CDCl_3$) : δ 153.03, 147.40, 147.28, 137.02, 133.61, 131.56, 109.82, 107.29(3C), 105.74, 101.19, 72.46, 72.20, 71.11, 62.24, 60.83, 56.23(2C), 49.12, 44.93, 42.99; Mass (m/z) : 460($M^+$), 399, 382, 321, 282, 247, 185, 115; **Compound 10** : mp : 85°C; IR : 3447, 1735, 1505, 1482, 1126, 1037, 795 $cm^{-1}$ ; $^1H$ NMR ($CDCl_3$, 200MHz) ; δ 7.14(s,1H), 6.48(s, 1H), 6.20(s,2H), 5.98(s, 2H),4.61(d, J=4.5Hz,1H), 4.30-4.17(m,2H), 3.91-3.68(m,2H), 3.86(s,3H), 3.78(s,6H), 3.72(s,3H), 2.90-2.38(m,3H), 2.30(br s, 1H, $D_2O$ exchangeble), 2.25-2.05(m.1H); Mass(m/z) : 472($M^+$), 441, 421, 398, 363, 321, 213, 181, 163; **Compound 13**: mp : 125°C : IR : 3249, 1709, 1613, 1482, 1248, 1114, 1037 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 200 MHz) : δ 6.90(s,1H), 6.40(s,3H),5.95(s, 1H),5.94 (s,1H), 5.50(s, 1H, $D_2O$ exchangeble), 4.80(d, J=4.6Hz, 1H), 4.15-3.70(m,4H), 3.85(s,6H), 2.95-2.78(m,1H), 2.63-2.23(m,3H), 2.15(s, 3H) ; $^{13}C$ NMR($CDCl_3$) : δ 207.33,147.15(2C), 147.05, 146.09, 133.53, 133.12, 132.85, 132.69, 108.92,106.87, 105.40 (2C), 100.88, 81.45, 69.10, 68.59, 56.30(2C), 49.64, 48.23, 46.05, 44.60, 30.57; Mass(m/z) : 442($M^+$), 424, 394, 366, 325, 288, 200, 167, 115.

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