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# NOVEL D-RING ANALOGUES OF PODOPHYLLOTOXIN AS POTENT ANTI-CANCER AGENTS#

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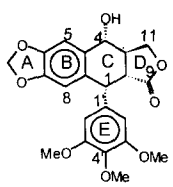
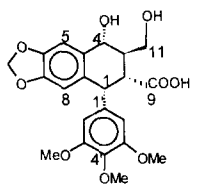
***Abstract :*** *Several D-ring modified analogues of podophyllotoxin were prepared viz semi-synthesis starting from naturally occuring podophyllotoxin and determined their in vitro anti-cancer activity. Most of the analogues have shown good activity towards human cancer cell lines.* © 1998 Elsevier Science Ltd. All rights reserved.

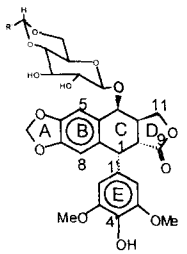
Among the various lignans isolated so far from the plant sources, podophyllotoxin**1**, has emerged as a lead plant toxin that inhibits the assembly of microtubules[1]. As the compound **1** was found to be highly cytotoxic for its clinical use against human cancers[2], extensive structural modifications of **1** have been undertaken which culminated into two semi-synthetic analogues of podophyllotoxin, namely, etoposide and teniposide. Although, these two compounds were widely used as anti-cancer drugs for small cell lung cancer, efforts for improving their clinical efficacy by overcoming the drug resistance, myelosuppresion and poor bioavailability problems[3] associated with them, were continued to be challenging. Consequently, the number of analogues of **1** increased considerably thereby the structural requirements for the better pharmacokinetic profile of podophyllotoxin **1** has become increasingly difficult[4].



**1** : Podophyllotoxin



**2** : Podophyllic acid



R' = Me , Etoposide

R' =  , Teniposide

Most of these analogues prepared so far have the D-ring lactone intact. The number of analogues having D-ring lactone opened were limited presumably because the *trans* fused-γ-lactone[5] was considered to be one of the essential features for these type of lignans to retain their anti-cancer activity. However, ethyl hydrazide derivative of podophyllic acid **2** (SP-1)[6] was found to possess potent anti-mytotic activity and its clinical efficacy was examined for some time and discontinued later due to severe side effects[7]. The importance of these compounds has once again gained momentum because of the recent discovery showing

#DRF's Publication No. : 31___Dedicated to my mentor Professor Goverdhan Mehta.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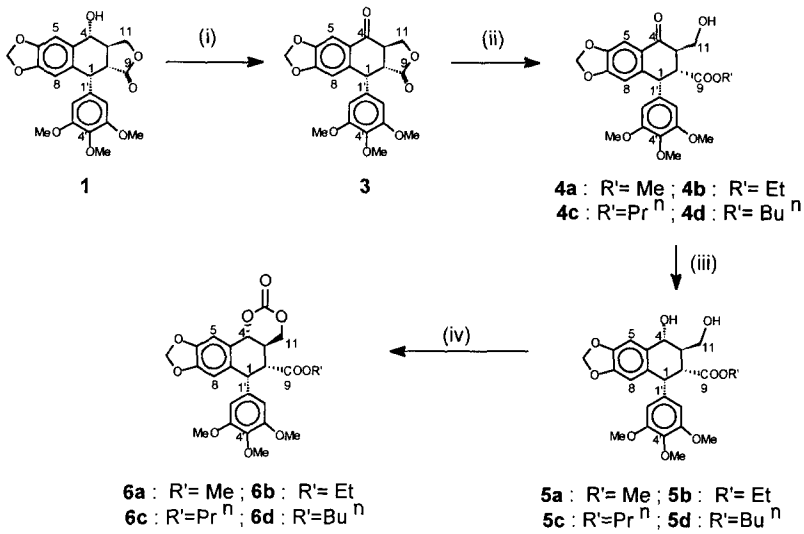
that the D-ring modified podophyllotoxins[8] were found to possess immunosuppressive activities. Therefore, we were also interested in investigating certain D-ring analogues of podophyllotoxins prepared semi-synthetically starting from naturally occuring podophyllotoxin **1**. Some of these derivatives were found to be extremely potent against human colon and breast cancer cell lines. The synthesis and their in vitro anti-cancer activity of these analogues were reported herein.

Podophyllotoxin **1** was oxidised to podophyllotoxone **3** using pyridinium dichromate (PDC). Lactone ring opening of compound **3** was carried out in different alcohols under strong acid conditions to

**Scheme 1**

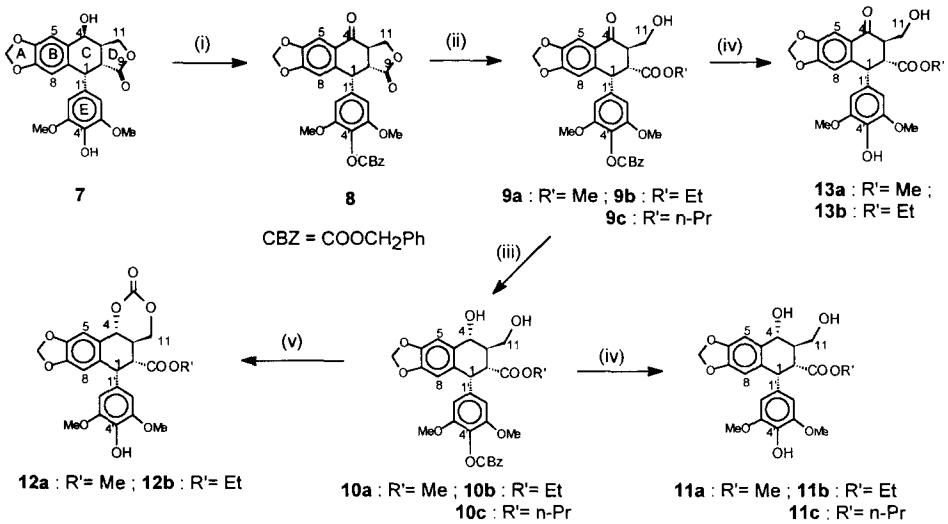



Reagents :(i) PDC, DCM, rt, 2h, 80% ; (ii) R'-OH where R'= Me, Et, n-Pr or n-Bu, $H_2SO_4$, 60°C, 45min., 60-65% ;(iii) $NaBH_4$, MeOH, rt, 0.5h, 85% ;(iv) 1,1-carbonyldiimidazole, $C_6H_6$, 80°C,70%;

furnish the corresponding esters **4a-4d** in 60-65% yield, Scheme 1. Stereoselective reduction of ketone in **4a-4d** was achieved with sodium borohydride to obtain exclusive formation of C-4α hydroxy ester **5a-5d**[9] in 85% yield. Reaction of the esters **5a-5d** with carbonyldiimidazole gave 4,11-carbonates **6a-6d**. Similarly, 4'-hydroxyderivative of the carbonates **12a,12b** were also prepared from 4'-demethyl epipodophyllotoxin **7** as shown in Scheme 2.

Protection of phenoxy group in **7** followed by the oxidation of C-4 hydroxyl group gave the ketone **8**. Opening of lactone moiety in **8** using alcohol and sulfuric acid gave the corresponding esters **9a-9c** in 65% yield. Debenzylation of these esters produced their 4'-hydroxy derivatives, Scheme 2. Stereoselective reduction of ketone in **9a-9c** using sodium borohydride provided C-4α hydroxyesters **10a-10c** respectively. Hydrogenation of **10a-10c** with 10% Pd/C afforded the corresponding C-4' hydroxyl derivatives **11a-11c**.

**Scheme 2**




Reagents : (i) Py, DCM, $ClCOOCH_2Ph$, rt ; PDC, DCM, rt, 2h, 80% ; (ii) R'-OH where R'= Me, Et, n-Pr, $H_2SO_4$, 60°C, 45min. 65% ; (iii) $NaBH_4$, MeOH, rt, 0.5h, 85°C ; (iv) $H_2$ / 10%Pd-C, EtOAc, 70% ; (v) 1,1-carbonyldiimidazole, $C_6H_6$, 80°C, 75% ; $H_2$ / 10%Pd-C, EtOAc, 60%.

**Table 1** : *In vitro* cytotoxicity (GI 50) data of podophyllotoxin analogues against colon cancer cell lines :

| COMPOUND | COLON CANCER CELL LINES | | | | | | |
|---|---|---|---|---|---|---|---|
| | COLO 205 | HCC 2998 | HCT 116 | HCT 15 | HT 29 | KM 12 | SW 620 |
| 4a | 7.71 | 10.8 | 6.26 | 5.71 | 3.52 | 3.62 | 3.71 |
| 4b | 2.22 | 5.63 | 4.35 | 3.99 | 3.36 | 4.21 | 2.07 |
| 5a | 0.01 | 0.03 | 0.04 | 0.04 | 0.02 | 0.02 | 0.02 |
| 5b | 14 | 14 | 24 | 13 | 17 | 8.4 | 26 |
| 5c | 1.2 | 1.7 | 0.42 | 1.4 | 0.89 | 0.56 | 0.7 |
| 5d | 1.9 | 1.8 | 3.7 | 2.0 | 2.5 | 1.86 | 2.2 |
| 11a | 0.1 | 0.8 | 0.2 | 1.02 | 0.09 | 0.11 | 0.34 |
| 11b | 1.16 | 1.3 | 1.7 | 2.07 | 0.88 | 0.78 | 1.17 |
| Etoposide | 18.1 | 3.46 | 6.16 | ---- | 15.13 | 5.62 | --- |
| 1 | --- | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 | --- |

All the above values were given in μM concentrations. The term GI 50 stands for the concentration of the drug that produced 50% growth inhibition (GI50) of the cells in the cell line under study.

Finally, reaction of the esters **10a,10b** with 1,1-carbonyldiimidazole followed by hydrogenation provided the 4'-hydroxycarbonates **12a,12b**[10].

**Table 2 :** *In vitro* cytotoxicity (GI 50)data of podophyllotoxin analogues against breast cancer cell lines :

| COMPOUND | BREAST CANCER CELL LINES | | | | | | |
|---|---|---|---|---|---|---|---|
| | MCF-7 | MCF7-ADR | MDA-MB 231 | HS 578T | MDA-MB 435 | MDA-N | BT-549 |
| 4a | 4.11 | 17.5 | 16.5 | 1.89 | 1.64 | 2.24 | 29 |
| 4b | 3.86 | 5.14 | 5.99 | 1.56 | 1.33 | 1.70 | 5.45 |
| 5a | 0.03 | 0.04 | 0.03 | 0.01 | 0.01 | 0.01 | 0.06 |
| 5b | 23 | 14 | 18 | 27 | 4.4 | 3.5 | >30 |
| 5c | 0.8 | 3.2 | >30 | 2.13 | 0.4 | 1.45 | >30 |
| 5d | 2.0 | 5.6 | >30 | ---- | 1.6 | 11.5 | >30 |
| 11a | 0.3 | 0.1 | 0.1 | 3.12 | 0.1 | 0.1 | 0.5 |
| 11b | 2.17 | ---- | 6.78 | 1.2 | 0.84 | 1.06 | 4.91 |

All the above values were given in μM concentrations. The term GI 50 stands for the concentration of the drug that produced 50% growth inhibition (GI50) of the cells in the cell line under study.

***In Vitro*** **Cytotoxicity** : Most of the compounds were tested at National Cancer Institute(NCI), Bethesda, USA for *in vitro* anti-cancer activity against 60 human tumor cell line assay. Some of the compounds were tested at our in-house facility against 6 human cancer cell lines taking one cell line from each cancer subtype following NCI's *in vitro* assay protocol[11]. Based on the data obtained from NCI, the ketones **4a, 4b** were found to be active whereas their 4'-hydroxyderivatives **13a** and **13b** were completely inactive even at 100μM conc.. In the case of 4,11-diols, all the compounds **5a -5d, 11a** and **11b** showed impressive activity against most of the colon and breast cancer cell lines as shown in tables 1 and 2. Among these esters, compound **5a** showed superior activity than the corresponding 4'-hydroxyderivative **11a**. However, in the case of ethyl ester, compound **5b** is much less potent than the corresponding 4'-hydroxyderivative **11b**. Surprisingly, the D-ring lactone opened diol derivative **5a** is equipotent to podophyllotoxin **1** in most of the cell lines tested, tables 1 and 3. However the other diol derivatives **5c, 5d, 11a,** and **11b** are less potent than **1** but more potent than etoposide, Table 1. These results shows that the D-ring lactone of podophyllotoxin **1** is not essential for its activity. Moreover, these compounds showed good sensitivity towards colon and breast cancer cell lines as shown in Tables 1 and 2.

Table 3 presents the *in vitro* cytotoxicity data of D-ring lactone opened diol-esters and the corresponding cyclic carbonates along with podophyllotoxin **1**, podophyllic acid **2** and etoposide. Etoposide and podophyllic acid **2** did not show impressive *in vitro* activity except against ovarian and melanoma cell lines. Both the diol-esters and the 4,11-cyclic carbonates have showed good activity in most of the cell lines in comparison to podophyllic acid **2**. When we compare the activity of open diols *vs* the corresponding carbonates, **6b** and **12b** showed improved activity than the diols **5b** and **11b** respectively, table 3. Overall the compound **5a** showed exceptionally better activity in all the cell lines. Since these compounds have C-4

substitution in α configuration just as in podophyllotoxin **1**, they might show tubulin binding properties. Evaluation of these characterstics and other pharmocokinetic studies of these derivatives are in progress.

**Table 3 :** *In vitro* cytotoxicity(GI 50) data of D-ring analogues of **1** :

| Compound | CANCER CELL LINES | | | | | |
|---|---|---|---|---|---|---|
| | SK-OV3 | MCF-7 ADR | DU-145 | A 498 | H 522 | M-14 |
| 5a | 0.03 | 0.04 | 0.04 | 0.03 | 0.01 | 0.04 |
| 11a | 0.34 | 0.1 | 0.47 | 0.40 | 0.10 | 0.86 |
| 5b | 29.9 | 14.3 | >30 | >30 | --- | 14.9 |
| 11b | 3.35 | --- | 1.63 | 1.10 | 0.1 | 2.1 |
| 6a | 2.0 | 20 | >30 | 10 | >30 | 70 |
| 6b | 1.5 | 8.0 | 0.09 | 0.2 | 2.0 | 6.0 |
| 6c | 0.9 | 2.0 | 0.5 | 0.3 | >30 | 2.0 |
| 12a | 0.2 | 1.0 | 0.5 | 0.4 | >30 | 0.4 |
| 12b | 30 | 1.5 | 0.6 | 0.4 | 0.2 | 0.2 |
| 1 | 0.9 | 0.6 | 0.04 | 0.08 | 0.7 | 0.07 |
| 2 | < 0.01 | 55 | 40 | >100 | 50 | < 0.01 |
| Etoposide | < 0.01 | 25 | 2.0 | 8.0 | 0.9 | < 0.01 |

All the above values were given in μM concentrations. The term GI 50 stands for the concentration of the drug that produced 50% growth inhibition (GI50) of the cells in the cell line under study. Representative cancer cell lines are Ovarian (SK-OV-3), ADR resistant Breast cancer(MCF7-ADR), Prostate(DU-145), Renal(A 498), Lung (H 522) and Melnoma (M-14).

In summary, a number of D-ring analogues of podophyllotoxin **1** are prepared from naturally occuring podophyllotoxin **1** and their *in vitro* anti-cancer activity was determined. The fact that most of these compounds have shown good activity towards human cancer cell lines empahasise that the open D-ring lactone derivatives of podophyllotoxin too have potential for pursuing further studies towards the development of better drug candidate. *In vivo* efficacy study of some of these compounds is in progress.

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9) A patent application no. Fr. 1,395,088, April 9, **1965**, by Sandoz Ltd., describing the preparation of podophyllinic acid esters was appeared in Chemical Abstracts : *CA*, **1965**, 63, 5653.; According to this reference, podophyllinic acid methyl ester was prepared from **1** by methanolysis using $ZnCl_2$.

10) All the new compounds have satisfactory analytical data. Spectral data of selected compounds are given here : Compound **4b** : mp : 86-88°C; IR : 3515,1738,1669, 1590, 1479, 1248, 1126 $cm^{-1}$; $^1H$ NMR ($CDCl_3$,200MHz) ; δ 7.52(s,1H), 6.58(s,1H), 6.13(s,2H), 6.04(s,1H), 6.02(s,1H), 4.57(d, J=5Hz,1H), 4.20(dd, J=11Hz, 2Hz, 1H), 4.07(q, J=8Hz, 2H), 3.78(s, 3H), 3.72(s, 6H), 3.57(dd, J=6Hz, 2H), 3.15-3.00(m,1H), 1.20(t,J=7Hz, 3H) ; Compound **6c** : mp : 210°C ; IR : 1764, 1720, 1484, 1220, 1130, 1037 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 200MHz : δ 7.10(s,1H), 6.41(s, 1H), 6.14(s, 2H), 5.96(s, 2H), 5.19(d, J=10Hz,1H), 4.80(dd, J=10Hz, 6Hz, 1H), 4.49(d, J=6Hz, 1H), 4.17(t, J=12Hz, 1H), 3.86(t, J=7Hz, 2H), 3.80(s,3H), 3.74(s, 6H), 3.05(dd, J=12Hz, 6Hz, 1H), 3.0-2.80(m,1H),1.51(q, J=7Hz, 2H), 0.84(t, J=7Hz,3H); Compound **11b** : mp : 220°C; IR : 3524, 1704, 1612, 1486, 1227, 1115, 1039, $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 200MHz) : δ 7.07(s, 1H), 6.38(s, 1H), 6.25(s, 2H), 5.91(s, 2H), 5.40(br s, $D_2O$ exchangeble,1H), 4.77(d, J=8Hz,1H), 4.29(d, J=6Hz,1H), 4.18-3.92(m,3H), 3.79(s, 6H), 3.80-3.62(m,2H), 2.97(dd, J=12Hz, 6Hz, 1H), 2.58-2.40(m,1H), 1.16(t,J=7Hz, 3H) ; Compound **12a**: mp : 180°C ; IR : 3512, 1750(br), 1485, 1244, 1218, 1121, 1036, 779 $cm^{-1}$ : $^1H$ NMR ($CDCl_3$,200MHz) : δ 7.10(s,1H), 6.41(s, 1H), 6.15(s, 2H), 5.95(s, 2H), 5.50(s, $D_2O$ exchangeble,1H), 5.20(d, J=10Hz,1H), 4.80(dd, J=10Hz, 6Hz, 1H), 4.48(d, J=6Hz, 1H), 4.15(t, J=12Hz, 1H), 3.80(s,6H), 3.57(s, 3H), 3.07(dd, J=12Hz, 6Hz, 1H), 2.95-2.70(m,1H) .

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